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Advisory Committee on Genetic Modification

COMPENDIUM OF GUIDANCE FROM THE HEALTH AND SAFETY COMMISSION'S ADVISORY COMMITTEE ON GENETIC MODIFICATION

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This is guidance prepared, in consultation with HSE, by the ACGM which was appointed by the Health and Safety Commission as part of its formal advisory structure. The guidance represents what is considered to be good practice by the members of the Committee. It has been agreed by the Commission. Following this guidance is not compulsory and you are free to take other action. But if you do follow this guidance you will normally be doing enough to comply with the law. Health and safety inspectors seek to secure compliance with the law and may refer to this guidance as illustrating good practice.

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Genetic engineering 13140

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COMPENDIUM OF GUIDANCE FROM THE HEALTH AND SAFETY COMMISSION'S ADVISORY COMMITTEE ON GENETIC MODIFICATION

Introduction

1. The Advisory Committee on Genetic Modification (ACGM) was formed in 1984, succeeding the Genetic Manipulation Advisory Group (GMAG). (The term "Manipulation" which was originally used in ACGM's title, was changed in 1990 to "Modification".) ACGM consists of an independent Chairman, representatives of employers, employees, informed lay persons and scientific and medical specialists. Members are appointed for three-year periods.

2. ACGM's primary responsibility is to advise the Health and Safety Commission (HSC) and the Health and Safety Executive (HSE) on the human health and safety aspects of the contained use of genetically modified organisms (GMOs). It also advises Ministers with environmental responsibilities in other government departments on the environmental aspects of such work.

3. ACGM was last reconstituted in 1996. This reconstituted Committee is different from its predecessors. It will address strategic and policy questions without, as previously, needing to devote substantial time and effort to the more narrowly technical questions associated with individual activities or with broader issues.

4. ACGM's new Technical Sub-Committee (TSC) will cover the sorts of technical issues previously discussed by ACGM itself and it will replace the *ad hoc* working groups to which detailed work on such issues was often remitted.

Legislation in outline

5. Activities involving GMOs in contained conditions are subject to the **Genetically Modified Organisms (Contained Use) Regulations 1992 (as amended by the Genetically Modified Organisms (Contained Use) Regulations 1996)**, which were made under the Health and Safety at Work etc. Act 1974 (the HSW Act). Assessments of environmental risks associated with genetically modified animals and plants are covered by section 108(1)(a) of the Environmental Protection Act 1990 together with the Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) Regulations 1996, as amended in 1997. Releases to the environment and marketing of GMOs are covered by a separate set of Regulations, the **Genetically Modified Organisms (Deliberate Release) Regulations 1992 (as amended in 1995)**, which were made under the Environmental Protection Act 1990. Together, these sets of Regulations implement within Great Britain parallel EC Directives on the contained use and deliberate release of GMOs. The Contained Use and Deliberate Release Regulations 1992 both came into force in February 1993, replacing and extending the 1989 Genetic Manipulation Regulations .

Approved Method

6. The Contained Use Regulations require containment measures for all Group II/Type A activities (see "A guide to the Genetically Modified Organisms (Contained Use) Regulations 1992, as amended in 1996" for a full explanation of these terms) to be determined by a method approved by the Health and Safety Executive. This is in addition to observing the principles of good microbiological practice and good occupational safety and hygiene (see Part 3 for further guidance). Details of the approved method are in Part 3 of this compendium of guidance.

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ACGM compendium of guidance

7. The 1992 Contained Use Regulations and Deliberate Release Regulations meant that some of the ACGM/HSE/DOEGuidance Notes were out of date or obsolete; several Notes also pre-dated the 1989 Regulations. Therefore, in 1993 the guidance notes were reprinted and re-issued as a compendium of guidance. This was welcomed by users and has been repeated in this edition of compendium.

8. The 1996 ACGM Compendium has been re-ordered as a series of interlocking sections covering general guidance (Part 1), guidance on risk assessment (Part 2) and guidance on containment and control measures (Part 3) - see the Contents page. **This edition of the Compendium replaces all of the guidance in the previous compendium, and in particular the separate ACGM/HSE/DoE guidance notes numbers 1 to 11. All of the earlier guidance should be considered to be withdrawn.**

Further guidance

9. The full text of the Contained Use Regulations, accompanied by comprehensive guidance, is contained in an HSE booklet "A Guide to the Genetically Modified Organisms (Contained Use) Regulations 1992, as amended in 1996" (ISBN 0-7176-1186-8, price £10.50). The Guide is available from branches of Dillons Bookstores or by mail order from HSE Books, PO Box 1999, Sudbury, Suffolk CO10 6FS. Tel: 01787 881165 Fax: 01787 313995.

10. A free leaflet, "Contained Use of Genetically Modified Organisms", ref IND(G)86(L)(rev), which summarises the main requirements of the Regulations, is also available from HSE Books.

Glossary and abbreviations.

The following abbreviations are used throughout the following compendium.

BSO	Biological Safety Officer
COSHH	Control of Substances Hazardous to Health Regulations 1994
DOE	Department of the Environment
GMM	genetically modified micro-organism.
GMO	genetically modified organism.
GMSC	Genetic Modification Safety Committee
HSE	Health and Safety Executive
MAFF	Ministry of Agriculture, Fisheries and Foods
MHSWR	Management of Health & Safety at Work Regulations 1992
SOAFD	Scottish Office Agriculture and Fisheries Department

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Addresses

General or technical advice relating to this guidance, notifications under the Contained Use Regulations or risk assessment can be obtained from the address below:

Health and Safety Executive
Directorate of Science and Technology
Unit E4
Magdalen House
Stanley Precinct
Bootle L20 3QZ
Tel: 0151-951-4772
Fax: 0151-922-7918

For general advice on policy issues relating to the legislation and including European developments:

Health and Safety Executive
Biotechnology Policy Section
Health Directorate B2
Floor 6 NW
Rose Court
2 Southwark Bridge
London SE1 9HS
Tel: 0171-717-6348
Fax: 0171-717-6199

For information on the Genetically Modified Organisms (Deliberate Release) Regulations contact:

Department of the Environment
Biotechnology Unit, Room A324
Romney House
43 Marsham Street
London SW1P 3PY
Tel: 0171-276-8187
Fax: 0171-276-8333

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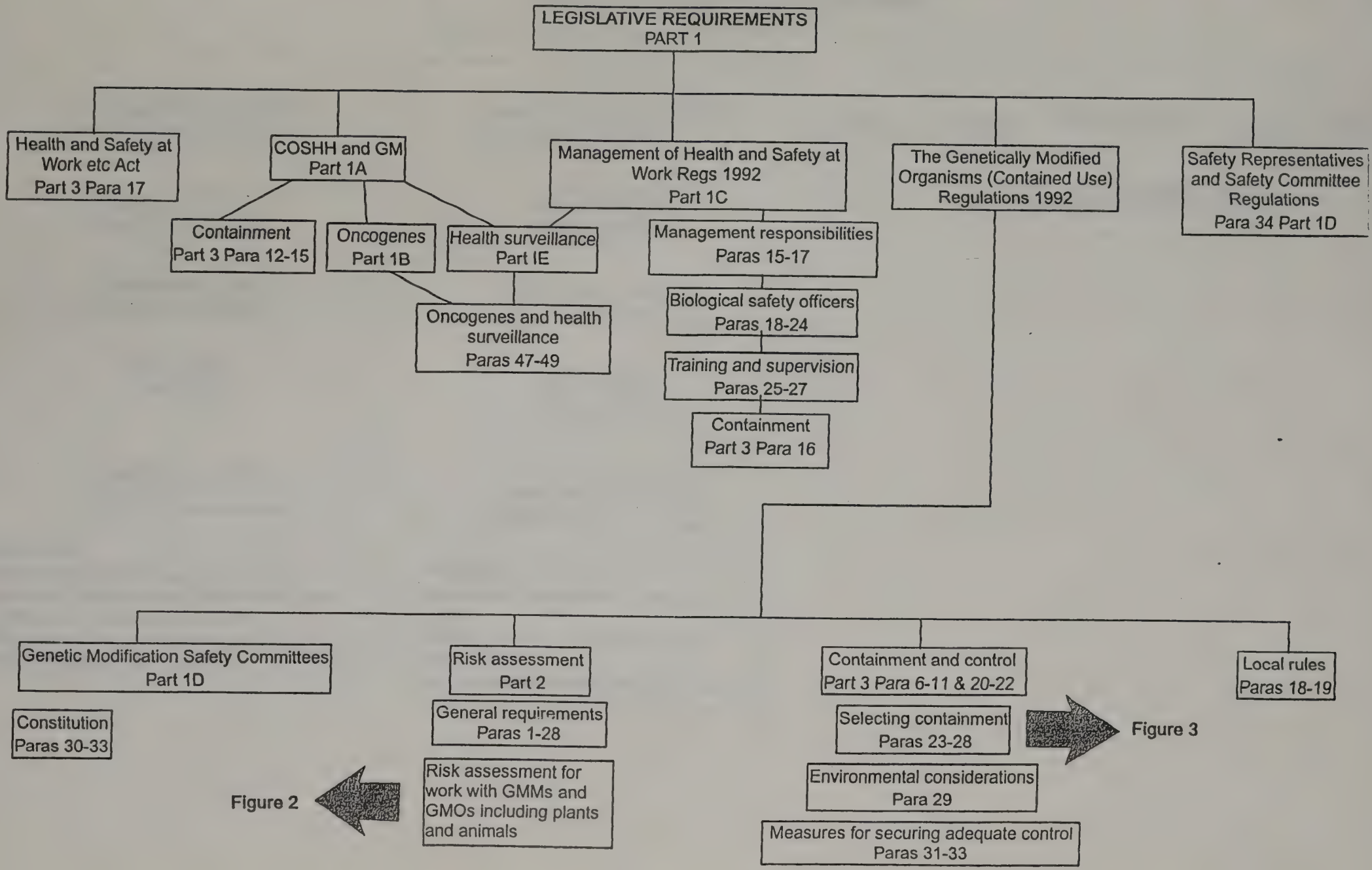

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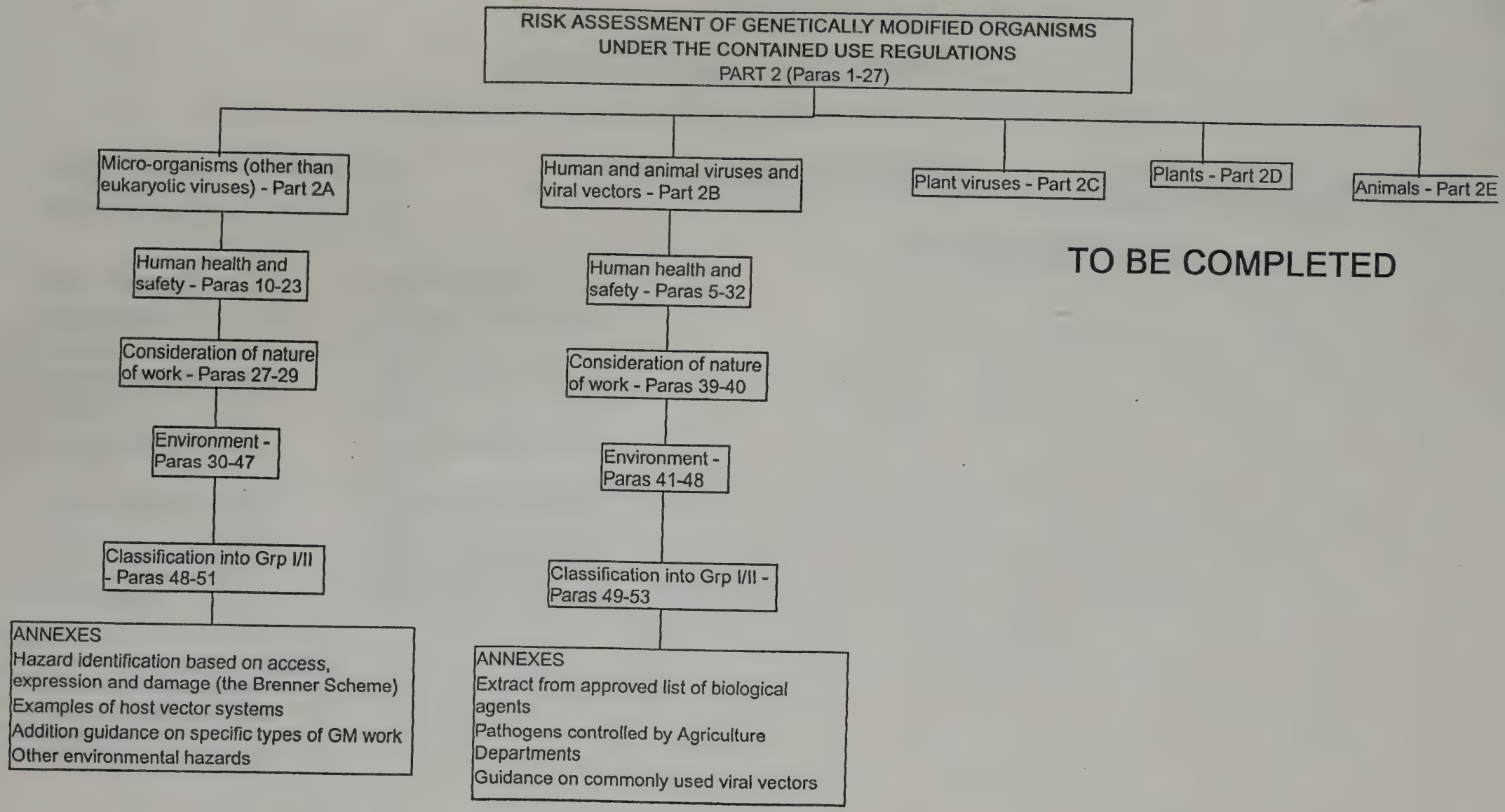
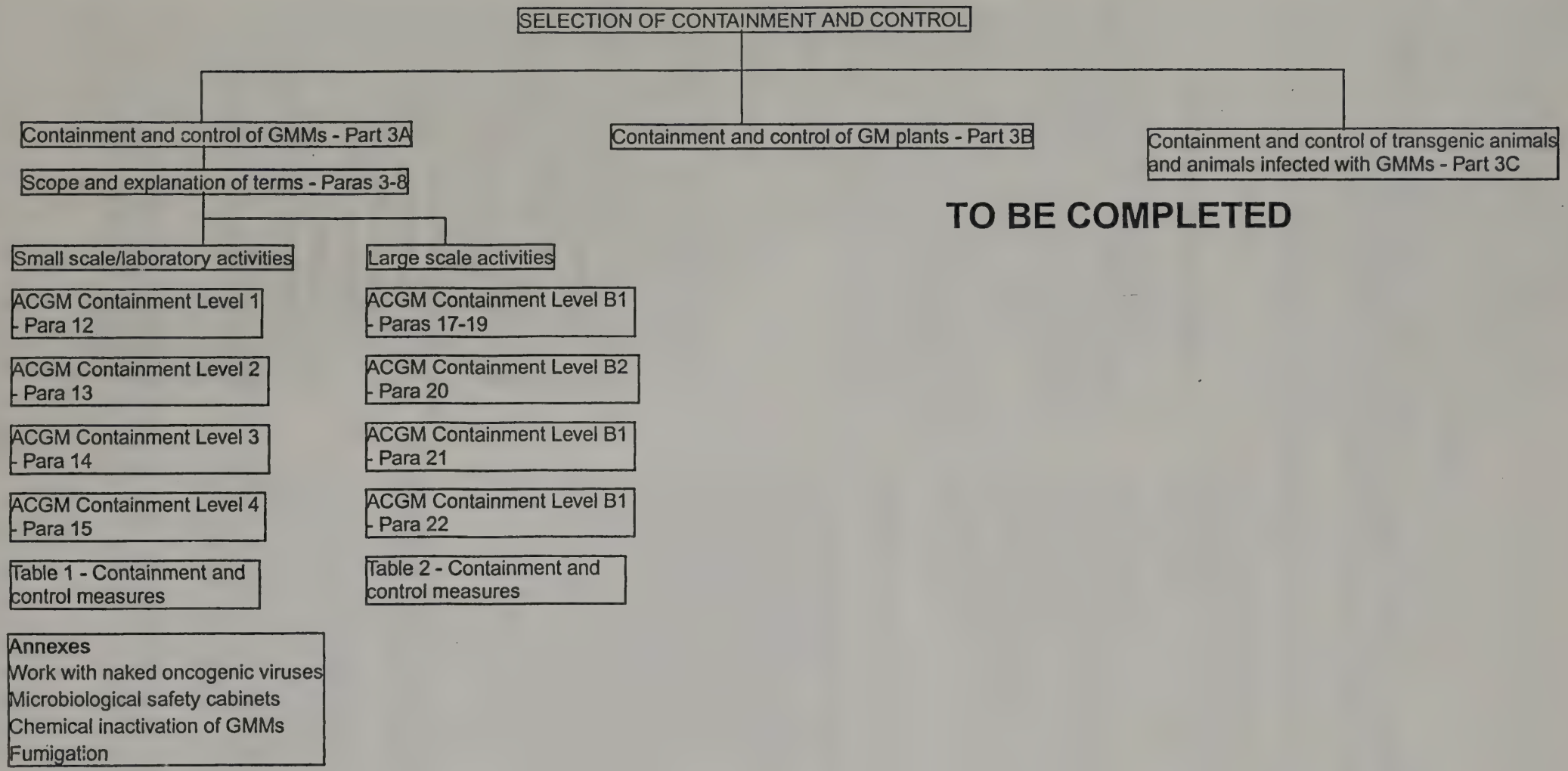

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TO BE COMPLETED

PART 1

APPLICATION OF OTHER HEALTH AND SAFETY LEGISLATION TO WORK INVOLVING GENETIC MODIFICATION

Part 1A

Genetic modification and COSHH.

1. The requirements of the Control of Substances Hazardous to Health (COSHH) Regulations[1] 1994 and the associated Approved Codes of Practice[2] (ACoP) may apply to certain GM work. The relationship between the Contained Use Regulations, COSHH, the General ACoP and the Biological Agents ACoP are discussed below. Additional guidance on biological agents can be obtained from the 4th edition of the publication "Categorisation of biological agents according to hazard and categories of containment"[3]

Biological Agents.

2. COSHH 1994 explicitly covers biological agents ("any micro-organism, cell culture, or human endoparasite... which may cause any infection, allergy, toxicity or otherwise create a hazard to human health") including any that have been genetically modified. It should be emphasised that not all GMMs will be biological agents, but only those which may present a hazard to human health. COSHH does not consider environmental risks. Containment and control measure requirements for a particular organism and in a particular activity may therefore differ between the two sets of legislation. However, the standards set in the four containment levels described in ACGM guidance on containment and control measures are broadly equivalent to those in COSHH (there are small differences which arise as GM legislation also covers the environment). Further details are given in Part 3 of the Compendium.

3. It is worth emphasising the general principle expressed in paragraph 9 of the Biological Agents ACoP; that where parts of the assessment required for COSHH Regulation 6 are carried out for the purposes of the Contained Use Regulations, they will not need to be repeated.

Prevention of exposure to a biological agent.

4. The biological agents provisions of COSHH introduce a requirement to, where reasonably practicable, prevent exposure to a biological agent by not using one, but if this is not possible by substituting a biological agent which is less hazardous (paragraph 5, Schedule 9). For work with many types of GMMs, this can be interpreted as a requirement, where possible, to use disabled or attenuated derivatives of pathogenic host and vector organisms. Further guidance on such systems for bacterial and eukaryotic viral vectors can be found in Parts 2A and B.

The GMM Group I/II classification scheme and biological agents Hazard Groups 1 to 4.

5. Once a GMM has been assigned to one of the four containment levels, and classified as required by the Contained Use Regulations 1992, this can be taken as also fulfilling the classification requirements under Regulation

[1] SI 1994 No 3246

[2] General COSHH ACoP and Carcinogens ACoP and Biological Agents ACoP, HSE Books 1995, ISBN 0 7176 0819 0

[3] Advisory Committee on Dangerous Pathogens, Categorisation of biological agents according to hazard and categories of containment HSE Books 1995 ISBN 0-7176-1038-1

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7(10) and Schedule 9 of COSHH. Similarly a notification made under the Contained Use Regulations can be taken as also fulfilling any notification requirements under Schedule 9 para. 12 of COSHH.

6. It should be noted that, unlike the classification for biological agents, the Group I or Group II classification does not directly equate to a containment level. Appropriate containment and control measures must be determined based on the risk assessment and for Group II GMMs taking into account the approved method of containment (see Part 3A).

Part 1B
Oncogenes and COSHH

7. The following guidance should be considered as applying to all known and potentially oncogenic DNA sequences. It replaces the guidance previously contained in ACGM/HSE Note 1 which is now withdrawn.

8. DNA sequences are regarded as oncogenic if they are able to make cells tumorigenic. Induction of a growth advantage in cells in culture is a useful means of identifying and assaying oncogenes although such phenotypes are not always associated with tumorigenicity. Growth advantages conferred by oncogenes include growth at confluence, focus formation, growth in low serum medium, growth in suspension and immortalisation. Further details and examples are given in Part 2B.

9. Potentially oncogenic sequences, particularly where they are handled as preparations of naked DNA or in viral vectors with a human host range, may be carcinogens as defined under the COSHH regulations. There is considerable difficulty in precisely assigning oncogenes to the three categories set out in the Chemicals (Hazard Information and Packaging) Regulations, 1993. The nature of the hazard will depend on the combination of gene and control sequences, as well as on the form in which it is encountered (as naked DNA, in a bacterial host vector system or in a eukaryotic virus with a human host range). Nevertheless, some potentially oncogenic sequences will clearly fall into category 1 or 2 and a great many more will be in category 3. In keeping with paragraph 2 of the General COSHH ACoP, it is prudent to adopt a precautionary approach to work with potentially oncogenic sequences, irrespective of whether they are COSHH carcinogens or not.

10. For work involving COSHH carcinogens (category 1 or 2 above), the General COSHH ACoP and the Carcinogens ACoP detail the particular requirements for the prevention or control of exposure and other requirements such as training.

11. In cases where oncogenic sequences are present in GMOs compliance with the requirements of the Contained Use Regulations and the Biological Agents provisions of COSHH will satisfy the carcinogens requirements under COSHH.

12. Further guidance is given on work with naked oncogenic DNA in Annex III of Part 2A and on the specific requirements for health surveillance in Part 1E.

Part 1C
Management of Health & Safety at Work Regulations 1992

13. Sections 2(1) and 3 of the Health and Safety at Work etc. Act 1974 (HSW Act)[4] require employers to ensure, so far as is reasonably practicable, the health and safety at work of their employees and that others who may be affected by their undertaking are not exposed to such risks.

14. The Management of Health and Safety at Work Regulations (MHSWR) 1992 and associated ACoP[5] set out more explicit duties

[4] 1974 c.37

[5] Management of Health and Safety at Work. Approved Code of Practice, 1992 HMSO ISBN 0 11 886330 4

and measures needed to comply with the HSW Act. In particular, employers should appoint competent persons to assist them in complying with health and safety legislation. In the context of the Contained Use Regulations, this requirement may be satisfied by the appointment of a competent biological safety officer. Again, a risk assessment for the purposes of the Contained Use Regulations and COSHH will form the basis of the assessment required under the MHSWR. The MHSWR also have requirements relating to training.

Management responsibilities

15. It is management's ultimate responsibility to ensure that the working environment is safe. This duty of care cannot be delegated to the workers. However, employees also have a duty to take reasonable care for both their own health and safety and that of others who may be affected by their acts or omissions at work.

16. Management should monitor and take notice of the activities of the BSO and the genetic modification safety committee (GMSC) set up to advise them on risk assessment, as well as the use of appropriate risk management measures. They should also keep under review the policies and implementation of control measures and ensure that they are effective.

17. The Contained Use Regulations place a number of statutory duties on employers in relation to both human health and environmental safety. The full details are outlined in the regulations and guidance however, the following is a summary of the main duties:

- to undertake a risk assessment covering both human health and safety and environmental safety [the risk assessment for environmental safety for GMOs that are not micro-organisms - for example transgenic animals - must be made under the Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) Regulations 1996, as amended in 1997. (See Part 2)]. Whoever undertakes a risk assessment shall appoint a genetic modification safety committee to advise them.
- to ensure that adequate containment facilities and procedures are in place to minimise any risks to workers and the environment.
- to maintain and test containment equipment at appropriate intervals and where necessary to monitor for the presence of process organisms outside of containment.
- to provide adequate training commensurate with the level of risk.
- to formulate and implement local rules.
- to formulate and implement emergency plans and procedures.

Biological Safety Officers

18. Clearly the majority of requirements listed in para 17 will need to be delegated down to a local level as employers are unlikely to have all the relevant knowledge to meet these statutory requirements. To allow for this, the MHSWR require every employer to appoint one or more "competent" persons to assist them in undertaking the measures required to comply with the relevant statutory duties [MHSWR Reg. 6 (1)].

19. Where GM work is undertaken, this role has traditionally been undertaken by a biological safety officer (BSO).

20. Whether or not employers choose to appoint a BSO will depend on the establishment and how they choose to meet their statutory duties. However, the competent person they appoint must have sufficient training and experience or knowledge and other qualities to enable them properly to assist in undertaking the measures required to meet all of the relevant statutory provisions [MHSWR Reg. 6 (5)].

21. The law makes it clear that where a BSO is carrying out the function of a competent person, they must be allocated sufficient time and resources to do the job. As well as advising on the containment and training aspects of the work,

the BSO will normally be expected to advise on risk assessment and co-ordinate the notification procedures. In large institutions it may be impractical to have one person carrying out this role alongside other duties such as research and teaching. Whilst these tasks may be undertaken by one person in a small department/institution or company, larger organisations may need to consider appointing more than one person to undertake the role.

22. The appointed person(s) should ideally have experience of working within a containment laboratory or with similar practices, but the absence of such experience should not necessarily preclude the appointment of an individual who is otherwise well suited for the position. Appropriate training and technical assistance should be provided as necessary and deputising arrangements made. The deputising arrangements are particularly important if the BSO is involved in a particular project.

23. Examples of matters upon which the BSO may advise or assist the person responsible to enable them to meet the statutory requirements for work with GMOs, include:

- ensuring that local rules are drawn up and followed for the safety of personnel;
- aspects of training of personnel in appropriate microbiological practice (the level of training will depend on the level of work being undertaken);
- investigating accidents, spillage etc. in the laboratory and taking what action they consider necessary. Each incident and the action taken is to be recorded, together with the names of the personnel involved;
- the safe storage of modified organisms/ harmful or potentially harmful material and ensuring that records of these are kept;
- the appropriate transport of all modified organisms;
- ensuring that laboratories are appropriately disinfected at the end of an experiment or before the entry of maintenance personnel. Appropriate disinfection could range from swabbing down work surfaces to complete fumigation and will be dependent on the risk assessment;
- participating in locally organised inspections;
- methods for testing, when necessary, for the presence of viable process organisms outside the primary containment;
- ensuring that control measures and equipment are tested and maintained at appropriate intervals;
- ensuring appropriate waste disposal procedures are used;
- providing technical support to the GMSC on risk assessment and classification;
- ensuring all statutory notifications are made to HSE;
- informing employers of the published changes to Regulations;
- physical security of the laboratory.

24. Where a BSO is not able to advise and assist in all these areas, the employer should appoint other suitably qualified person(s) to enable them (the employer) to meet their statutory duties, for example, by using outside contractors to test and maintain microbiological safety cabinets.

Training and Supervision.

25. Training is required under the Contained Use Regulations and also by the MHSWR 1992. It can be divided into:

- training on recruitment, for example, training in good microbiological practice and familiarisation with the local rules before beginning work;
- training when a significant change to equipment, work environment or work activity takes place, especially where increased or novel risks may be involved;
- refresher training (where appropriate) to maintain standards;
- training in risk assessment procedures.

26. The level of training provided to staff should be appropriate to the level of risk or the complexity of the operations being undertaken.

In smaller groups or establishments training is often given on a one to one basis, with research supervisors demonstrating techniques, then progressing on to allow the new employee to perform the technique under supervision. Once satisfied with the competence of the worker, the supervisor finally allows them to continue without constant supervision. The length of time involved in this supervised training will clearly relate to the level of the work.

27. At containment level 3 and above, a more formal approach to training is appropriate, with written records of training kept. It may also be appropriate to keep formal training records for some containment level 2 projects, depending on the risk assessment. Usually staff commencing work at higher levels will be assessed for their suitability, for example their ability to work safely with non-pathogenic micro-organisms. At containment levels 3 and 4, all work with live organisms will be performed in a microbiological safety cabinet, so staff should be trained in the safe use of cabinets. Other aspects that should be covered include safe transport and storage of organisms and waste management procedures. New workers should be trained in emergency procedures, for example, what action to take in the event of accidental spillage of culture and should be familiar with disinfection and fumigation procedures.

Part 1D
Genetic modification safety committees

28. As stated above, the Contained Use Regulations place a statutory obligation on anyone carrying out a risk assessment under those regulations to establish a GMSC to advise on that risk assessment. The ACGM and HSE attach great importance to the safety committee, which often plays a key role in the organisation of safety procedures.

29. Although the statutory purpose is solely to advise on risk assessment, the GMSC can also usefully be involved in ensuring good practice and that there is full discussion with all those concerned, on safety, training and laboratory discipline. Members' local knowledge and expertise can be particularly important. GMSC's are often involved in the formation of local rules and in the consideration of accidents and incidents.

Constitution

30. There are no hard and fast rules governing the make-up of a GMSC. However, it should ideally be constituted to represent both management and employees with its members also being representative of all persons having access to the genetic modification facilities or who might otherwise be exposed to such work. It should have enough members, with sufficient depth and range of knowledge and experience to:

- understand the risks to both human health and the environment arising from the normal range of activities undertaken at the facility, and the extent to which any risks are uncertain;
- judge the adequacy of the risk assessment made under Regulation 7;
- test its emerging conclusions by discussion so that the advice given is genuinely that of a committee and not an individual.

31. ACGM places particular emphasis on the value of having a balanced committee representing both management and employees and the need for the committee to be run in such a way that all members' views are heard.

32. The appropriate composition for a GMSC will depend on local circumstances and may include representatives with some or all of the following functions:

- a Chairman, preferably elected by the committee;
- representatives of management with responsibility for the work in genetic modification;
- representatives, chosen by and from all persons having access to the genetic modification facilities or who might otherwise be exposed to genetic modification work,

e.g. technical and ancillary staff, students or visiting workers;

- the Biological Safety Officer (see guidance above on BSOs);
- where appropriate, someone to liaise between the GMSC and a main safety committee;
- co-opted members to supplement internal expertise where necessary, for example on specific viral vectors, medical or environmental considerations. Often these will be from another department, laboratory or outside body.

33. The membership of the GMSC must be notified to HSE as part of the notification of first use of premises for genetic modification activities. Any changes to the membership should also be notified as part of the Annual Return.

Safety Representatives and Safety Committees Regulations

34. The requirement for a GMSC under the Contained Use Regulations in no way affects the rights of safety representatives appointed under the Safety Representatives and Safety Committees Regulations 1977[6] (as amended by the MHSWR 1992) to request their employer to establish a safety committee under the 1977 Regulations. Such committees have the function of keeping health and safety measures under review, which could include measures relating to genetic modification. It is essential, therefore, that the relationship between any such committee and the GMSC is clearly defined. For example, the GMSC may be a sub-committee of the main safety committee. Local circumstances and the wishes of those represented on the main safety committee will have to be taken into account in determining the best arrangement. More recently, the Consultation with Employees Regulations 1996[7] have been introduced. These place a statutory duty on management to consult staff on issues which impinge on health and safety.

Part 1E
Health surveillance

35. This section outlines the legal requirements for health surveillance as they apply to people involved in genetic modification. It replaces the guidance previously contained in ACGM/HSE Note 4 which is now withdrawn.

36. The Contained Use Regulations do not include a specific requirement for health surveillance for GM work, but other more general regulations, such as the MHSWR and COSHH, will be applicable in certain cases. In interpreting the health surveillance requirements of the above Regulations users should consider whether the genetic modification aspects of the work involve a significant risk to health and whether health surveillance is appropriate (see below).

37. Regulation 5 of MHSWR 1992 states that "every employer shall ensure that his employees are provided with such health surveillance as is appropriate having regard to the risks to their health and safety which are identified by the [risk] assessment" and the ACoP contains further interpretation of general provisions for health surveillance if certain criteria are shown to apply to the work.

38. More specifically, COSHH regulation 11 requires health surveillance where it is appropriate for the protection of the health of workers exposed to a substance hazardous to health (which includes biological agents and, therefore, certain GMMs). Health surveillance is appropriate when:

- an identifiable health effect may be related to exposure; and
- there is a reasonable likelihood that the disease or effect may occur under the conditions of the work; and
- there are valid techniques for detecting indications of the disease or health effect.

[6] SI 1977/500 as amended by SI 1992/2051

[7] SI 1996/1513

39. Health surveillance may also be useful to assist in:

- the evaluation of the measures taken to control exposure;
- the collection of data to evaluate the hazards to health; and
- determining the immunity of workers to biological agents.

40. Paragraphs 82 to 97 of the General COSHH ACoP contain further interpretation of the health surveillance provisions and the Biological Agents ACoP (paragraphs 23 to 25) gives interpretation specific to work with biological agents. Further general guidance on health surveillance can be found in the HSE publications "Health surveillance under COSHH" (ISBN 0 7176 0491 8) and a free HSE leaflet entitled "Health surveillance in the pharmaceutical industry" (ref. IND(G)158(L)).

Low risk work with GMOs

41. For low risk work with Group I GMMs and for Group II GMMs with no identifiable risk to human health (e.g. many plant pathogens), it is very unlikely that there will be a need for health surveillance under COSHH etc.

42. There may, however, be some value in identifying workers who may be at greater risk because of pre-existing illness or an underlying medical condition. It is for local judgement and decision, in each instance, whether a particular medical condition would require additional control measures to protect the workers health. In common with other areas of microbiological risk, some general considerations that might be borne in mind are:

- any relevant medical history (e.g. history of asthma, recurrent infections);
- evidence of defective barriers to infection (disorders of skin, respiratory tract and alimentary canal);
- immune competence;
- treatment with antibiotics, especially those used in the experimental programme, or the therapeutic use of steroids; some forms of self-medication which could influence the chance of infection.

Higher risk work with GMOs

43. Where there is a risk of ill health resulting from work exposure to a GMM and there are methods available to detect disease, then some form of health surveillance may be necessary. Examples of possible hazards related to GM work that may be considered are:

- GMMs derived from biological agents classified in ACDP Hazard Groups 2 - 4, particularly for example, where modified viruses may exhibit different tissue tropism, or where the agent is less susceptible to therapeutic agents, or where immunised workers may not be fully protected (fuller guidance in Part 2B);
- cloning of oncogenic or tumorigenic sequences, mutant tumor suppressor genes or anti-sense constructs for tumor suppressor genes;
- work with modified prion protein genes;
- organisms expressing biologically active molecules such as enzymes, hormones, toxins which may pose risks to health;
- work with a potential for exposure to cloned human genes which may lead to an immune response and subsequent auto-immune type disease;
- work that may cause respiratory sensitisation, especially at large scale and with the possibility that fusion proteins or inclusion bodies may enhance sensitisation;

Health surveillance procedures.

44. The following health surveillance procedures are listed in the COSHH General ACoP and their application to GM work should be decided on a local basis. Users should note the important general considerations detailed above before adopting any of the following procedures.

- **Biological monitoring and biological effects monitoring.** There are a variety of monitoring procedures which may be

relevant for GM work, one example would be the use of serum samples as a means of determining immunity and the efficacy of vaccination (para. 23, Biological Agents ACoP) or to detect seroconversion indicating exposure. In the past ACGM guidance recommended that serum samples were kept to maintain a baseline reference. It should be noted that ACGM is not recommending this practice, nor is it a requirement of the legislation. Furthermore, the storage of serum may only be relevant in some specific circumstances. Before a decision is taken, the ultimate purpose of such a procedure should be considered. Long term, effective serum storage requires good organisation and record keeping and can be costly particularly for large academic departments. **Note:** It is also worth remembering that serum samples have only dubious value as a means of detecting a disease or adverse health effect where the GMO contains genes that are homologous to, or identical to, normal human genes. In addition, in the case of oncogenes there is considerable difficulty in detecting people susceptible to cancer or with the early stages of the disease.

- **Medical surveillance.** This is only one of several options where health surveillance is required, but there are no specific requirements for medical examinations of GM workers. Also note that there is no longer a general recommendation to appoint a supervisory medical officer (SMO) responsible for the continuous surveillance of the health of workers involved in GM work or to be an *ex officio* member of the local genetic modification safety committee.
- **Enquiries about symptoms.** This can include examination by a suitably qualified person or a medical health questionnaire to enquire about any symptoms that may be related to exposure to a hazardous GMO at work. In many cases, providing information about symptoms and encouraging self-monitoring and reporting of symptoms may also be effective health surveillance procedures.

Records of exposure

45. The COSHH Regulations (paragraph 11(3) of Schedule 9) and Biological Agents ACoP requires that records of exposure are kept for work with certain biological agents. These include all biological agents classified in Hazard Groups 3 and 4, for which records should be kept for 10 years after work ceases.

46. In some cases where there is a possibility of delayed onset of ill health, the records must be stored for 40 years. Such agents include hepatitis B, C, D and unclassified hepatitis viruses, human papillomaviruses, human retroviruses and the agents responsible for Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome and kuru. Further details can be found in the 4th edition of the ACDP Categorisation guidance.

- Because of the possibility of a novel aetiology of CJD, GSS and other 'prion diseases' and the corresponding uncertainty over the need for exposure records, ACGM feels that it is prudent to recommend that exposure records are kept for 40 years for all persons working with modified prion protein genes (see Part 2A, Annex III for further guidance on terms). The exception is where the risk assessment for the GMO and the intended work practices indicate that this is not necessary.

Work with oncogenes and other hazardous sequences

47. General considerations relating to oncogenic sequences are given in Part 1B. Work involving oncogenes, particularly where these are handled as preparations of naked DNA or viral vectors with a human host range, may be subject to special health surveillance requirements if they are considered to be carcinogens under the COSHH Regulations.

48. The Carcinogens ACoP and paragraph 92 of the General ACoP indicates that the collection, maintenance and review of health records will always be required for work with carcinogens, unless the risk assessment shows that the exposure is not significant. In view of the long term nature of the postulated risk from exposure to oncogenic and related sequences, ACGM recommends that health records should be kept

for work with oncogenes and related sequences, unless the risk assessment clearly indicates otherwise.

49. Health records for such work should include, as a minimum, the details in the appendix to the General COSHH ACoP. Specific consideration should also be given to an historical record of occupational exposure, which should include details of the project and oncogenes studied, the room number or identification of the laboratories worked in, the dates work started and finished. These records should be updated periodically, at least once a year would be appropriate, and should be kept for at least 40 years after work ceases. All such records should be stored securely. Upon termination of a contract, a copy of the records should be given to the worker so that they may be given to the next employer to form part of any records kept. This may be particularly important for researchers undertaking a number of short term contracts.

PART 2

RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS

Introduction

1. The Contained Use Regulations require that, before any premises are used for activities involving genetic modification (GM) for the first time, and subsequently before each new GM activity is started, an assessment is made of the risks to human health and safety and to the environment.

2. The following guidance has been prepared by the Health and Safety Executive (HSE) and the Department of the Environment (DOE) with the advice of the Advisory Committee on Genetic Modification (ACGM), its Working Groups and most recently its Technical Sub-Committee. It is intended to help users and Genetic Modification Safety Committees (GMSC) to assess the risks to human health and safety and to the environment from contained use operations with genetically modified organisms, and to make decisions about the appropriate containment and control measures.

3. **The guidance set out below gives a workable approach to risk assessment and classification, but it is not the only one. Users may adopt other approaches provided that the requirements of regulation 7 are satisfied and control measures are properly matched to human and environmental risk.**

4. As well as the general guidance on risk assessments in this section, there is detailed guidance on different GMOs and activities in the following sections:

- Part 1 for guidance on risk assessments under the COSHH Regulations;
- Part 2A on the risk assessment of work with bacterial and cell cultures (replacing ACGM Note 7);
- Part 2B on the risk assessment of work with genetically modified human and animal viruses (replacing ACGM Note 5);
- Part 2C on the risk assessment of work with genetically modified plant viruses (replacing part of ACGM Note 10);
- Part 2D on the risk assessment of work with genetically modified plants (replacing ACGM Note 10);
- Part 2E on the risk assessment of work with transgenic animals (replacing ACGM Note 9).

Risk assessments under the Contained Use Regulations

5. The Contained Use Regulations mention risk assessments in three ways:

- A "suitable and sufficient" risk assessment (required for all activities);
- A summary of the risk assessment (required with certain notifications - see below);
- A record of the risk assessment (to be kept for 10 years after the activity has ceased).

6. The risk assessment is everything that has to be considered in order to come to conclusions about the hazards of the organisms and activity, the likelihood that they will actually give rise to harm, and the control measures that are needed.

7. In general a risk assessment should involve the following elements:

- hazard identification;
- assessment of exposure to the hazard and the consequences of that exposure;
- assessment of the level of risk (by consideration of the magnitude of harmful consequences and likelihood of their being realised);

- selection and assignment of appropriate control measures (risk management).

8. A practical approach to the application of this general scheme that takes particular account of the issues involved in the assessment of micro-organisms is developed in Parts 2A and 2B.

9. The level of detail to be considered in a risk assessment will depend on circumstances. It has to be "suitable and sufficient" [regulation 7(1)]. However, this does not mean it has to be very detailed; it may be short, for example, where it is immediately obvious that the risks are low or that the proposed control measures are clearly adequate. For a simple operation involving a low hazard, well known and well understood organism it may be possible to declare the result of the assessment almost at first glance. For a complex operation involving dangerous organisms about which there is a lot of uncertainty, the assessment will have to be extensive and may involve the acquisition of new data.

10. Note that it is always permissible to assume the worst and act accordingly, if the cost of a more precise assessment is disproportionate. In other words, if there is a range of risk for a given organism and activity within which you are uncertain, you may simply choose to apply control measures appropriate to the upper bound.

11. A risk assessment should be reviewed if there is any reason to suspect that the initial assessment is no longer valid because of a significant change in the activity [regulation 7(4)]. Examples of such changes might include alteration in type of operation (Type A or B), scale, containment measures, waste treatment or the availability of new information concerning the GMO.

Risk assessment parameters - Schedule 3 to the Regulations.

12. In making an assessment of the risks to human health and the environment the user should take due account of the parameters in Schedule 3 to the Regulations in so far as they are relevant [regulation 7(3)]. This can be viewed as a means of obtaining and recording relevant information about hazards to either human health or the environment.

13. In general it will probably be only necessary to record briefly any relevant information as this will be expanded on when assessment of the level of human and environmental risk is made (see detailed guidance in Parts 2A, 2B etc.).

14. Schedule 3 requires that all of the components of the GMM should be considered, including the host (recipient) organism, the donor(s) from which any inserted DNA has been derived, the inserted DNA itself and any vector sequences. In many cases, the characteristics of the host organism will be more relevant to a risk assessment than those of the donor organism, eg, this will not be the case for cloning work with *E. coli* K12 strains.

15. As a general guide, if a donor organism is merely used as a source of well characterised DNA for a selectable phenotype (e.g. kanamycin resistance or ß-galactosidase activity) or a promoter or other control sequence, the characteristics of the donor will not need to be considered. If, however, the insert contains genes encoding biologically active molecules, toxins or virulence factors, then relevant information from the donor organism should be considered.

16. When constructing cDNA or genomic libraries it will be necessary to consider the range of the possible hazards associated with the donor organism. This should be commensurate with the level of hazard and the likely abundance of hazardous sequences.

Risk assessment for environmental protection

17. Before the introduction of the Contained Use Regulations, containment was assigned to operations solely to protect human health and safety. Now there is a requirement to protect the environment. For the very large majority of users however, containment sufficient to protect human health will also be sufficient to protect the environment.

18. Nevertheless, an environmental risk assessment is required to ensure that the containment indicated with respect to human health and safety reduces all of the identified environmental risks to an acceptable level.

19. If, after having carried out the environmental risk assessment, the level of containment set for human health and safety is judged not to be sufficient, the best approach is to identify which element of containment is lacking.

> For example, an unusual situation might occur where ACGM level 2 offered sufficient containment in all but one aspect (e.g. treatment of exhaust gases). The addition of this one facet of control would be deemed sufficient in terms of "BATNEEC"[1] for environmental protection and ACGM level 3 would not be necessary. Similarly, if insect spread of the GMM were identified as a hazard, the installation of a specific insect control device to trap and kill any flying insects before they entered the laboratory might be a sufficient control measure to protect the environment.

"Harm" to the environment and "risk"

20. "Harm to the environment" is difficult to define or to quantify precisely. Section 107(6) of the Environmental Protection Act 1990 (EPA) is useful as guidance, though the Act does not refer directly to the GMO (Contained Use) Regulations, 1992. The EPA defines harm as follows:

> "harm means harm to the health of humans or other living organisms or other interference with the ecological systems of which they form part and, in the case of man, includes offence caused to any of his senses or harm to his property".

21. For example, following an escape from containment, for instance of pollen from a glasshouse, or of transgenic animals, the unintentional transfer of genes to a farmer's crops or livestock is a potential hazard and could constitute harm if realised. Toxic or allergenic effects arising from the expression of genes in pollen is another potential hazard to human health.

22. The environment is defined as "land, air or water" and harm would result if an organism affected any or all of these components of the environment directly, or in such a way that in turn, deleterious effects were produced on other organisms ("knock-on" effects).

23. Components of the environment are here taken to mean organisms and systems of which these form part.

24. Risk is defined[2] as the probability that a particular adverse event (or "harm") occurs during a stated period of time or results from a particular challenge. In the context of the contained use of GMOs, therefore, the objective of an environmental risk assessment is to determine the probability of harm to the environment arising as a result of the escape of organisms from the containment facility. This assessment must include escape to the environment by means of waste streams/waste disposal etc.

25. Risk evaluation has to take into account an assessment of the degree of potential harm, and the likelihood, or frequency, of that harm occurring.

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[1] Best Available Technique Not Entailing Excessive Cost, Environmental Protection Act, 1990

[2] Risk: Analysis, Perception and Management: The Royal Society, London 1992

Risk assessments under the Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) Regulations 1996, as amended in 1997 and Part VI of the EPA 1990

26. Whilst the Genetically Modified Organisms (Contained Use) Regulations require users to carry out an environmental risk assessment for work with micro-organisms, they do not require such an assessment for non-micro-organisms, for example, for work with transgenic animals or plants. This is, however, covered by Part VI of the EPA 1990, and was brought into force by the Genetically Modified Organisms (Contained Use) Regulations 1993. These regulations were superseded by the Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) Regulations 1996, which require users to carry out an environmental risk assessment of operations with transgenic plants or animals with reference to the means by which they are contained. This assessment should be kept for 10 years and be available to inspectors on request.

27. Details of the type of information required is given in the sections on transgenic plants (2D) and animals (2E).

Summary of the risk assessment.

28. A summary of the risk assessment is required as part of any notification to enable the scrutineers of a notification to form a judgement about the risks of the proposed activity and the suitability of the intended controls. (It is, of course, permissible to send in the full risk assessment, instead of preparing a separate summary for the purpose of notification). It should be submitted as part of any notification of:

- 'first use';
- Group I Type B operations;
- Group II Type A operations; and
- significant changes in activity under regulation 10(4).

29. The full risk assessment should be submitted with notifications of Group II GMMs in Type B operations. How much is needed on any particular point will depend on its importance in the assessment and the extent to which it is generally accepted material. There is no need to include every detail or to spell out in detail what is in the text books or HSE/ACGM guidance, etc. But the logic of the argument should be clear and enough detail should be included for the assessment to be reviewed without needing to request additional information. Note also that under regulation 10(1) any delays caused by the need to request additional information will not be counted as part of the time period for review of a notification and, in addition, work may not commence until HSE has given its approval.

Examples of summaries of risk assessments can be found in ACGM Newsletter 17 issued in April 1994.

PART 2A

RISK ASSESSMENT OF GENETICALLY MODIFIED MICRO-ORGANISMS OTHER THAN EUKARYOTIC VIRUSES.

Introduction

1. This part is intended to provide guidance on the risk assessment, for human and environmental safety, of work with modified bacteria, fungi and cell cultures. It covers work with most types of cloned DNA, including oncogenes, proviral DNA and prion protein genes (see Annex III).

Structure of the guidance

2. The following procedure for risk assessment, and the assignment of containment and control measures is recommended:

(i) Consideration of the predicted properties of the GMM to determine if there are any potential mechanisms by which it could represent a hazard to human health.

(ii) Consideration of the likelihood that the GMM could actually cause harm to human health.

(iii) The assignment of the general controls necessary to safeguard human health i.e. the allocation of a provisional level of containment.

(iv) Consideration of the nature of the work to be undertaken.

(v) The identification of any hazards to the environment and then, on the assumption that the controls necessary to safeguard human health have already been applied, the assignment of any additional containment measures to protect the environment.

(vi) Classification into Group I/II.

One variation on this structure will be in situations where the ACGM scheme, using access, expression and damage[1] (see Annex I) forms the basis of the hazard identification process. In such situations the application of the scheme will substitute for parts (i) (ii) and (iii) above.

3. Many users will be familiar with the ACGM scheme which was used in earlier guidance, and applied to the protection of human health and safety. This method of assessment - generally known as the Brenner Scheme - has been in use since the early 1970's, and was designed for use at a time when work that was almost entirely based on cloning into *E. coli.* It essentially provides a method of determining whether a host strain might be made hazardous by cloning in a foreign gene.

4. The scheme considers three characteristics of the GMM, under the headings:

- Access - the likelihood that the organism could enter and survive in a human;
- Expression - a measure of the level of expression of the cloned protein;
- Damage - the potential for the expressed protein to cause harm.

5. Full details of this system are given at Annex I. It should be noted that these considerations do not constitute a comprehensive risk assessment, and only give an indication of the level of containment appropriate for human health.

There are many instances when consideration of Access, Expression and Damage does not give a reliable indication of the appropriate containment level. Examples of situations where this is the case include:

[1] Often referred to as "the Brenner Scheme".

- cloning of genes that alter or exacerbate existing pathogenic traits, e.g. pathogenicity determinants, or antibiotic resistance genes whose dissemination might prejudice clinical use of the antibiotic;
- work with host strains where there is uncertainty over the level of attenuation;
- work that does not involve a construct formed in a classical way, from a plasmid vector and an inserted coding sequence, e.g. deletion mutants, certain cell fusions.

6. In these cases containment is better assigned on the basis of a full assessment of the GMM, rather than the indicative level obtained using the ACGM scheme.

7. It is recognised that experience and knowledge of work with disabled *E. coli* has shown this work to be generally low risk, unless, for example, bacterial toxins are being cloned. Therefore most routine cloning work in attenuated hosts such as *E. coli* K12 will require only a brief assessment and, in many cases, human health risks can be assessed using the established Access, Expression and Damage scheme (see Annex I).

8. Much of the guidance in this Part deals with activities where there is some uncertainty and a more in-depth assessment is required. The level of detail for individual cases will be different, dependent on the nature of the hazards or level of scientific uncertainty. Where a potential for harm is identified, a more detailed consideration of the risks associated with the activity should be undertaken.

9. Appropriate containment and control measures must be assigned on the basis of both human health and environmental aspects of the risk assessment. In the majority of cases the containment and control measures appropriate to the protection of human health and safety will also be sufficient to protect the environment. This will be particularly so for work involving, for example, *Saccharomyces*, disabled or multi-auxotrophic strains of *E. coli* or well characterised mammalian cell lines, which are extremely unlikely to pose a risk to the environment.

Risk assessment for human health

(i) Consideration of the predicted properties of the GMM to determine if there are any potential mechanisms by which it could represent a hazard to human health.

10. Factors to consider during hazard identification are listed in Schedule 3 to the Contained Use Regulations. These include pathogenicity, the biological activity or toxicity of the foreign gene product and the mobility of plasmid or viral vectors.

(a) Hazards associated with the host/recipient

11. Consideration should be given to the pathogenicity of the host strain, including virulence, infectivity and toxin production. Where appropriate, allergenicity should be included. The presence of any harmful adventitious agents should also be considered, particularly when using cell cultures.

12. An estimation of the degree of pathogenicity of the host strain, and the seriousness of the consequences of exposure should be made. Where the recipient is an acknowledged human pathogen, the organism should be assigned to the hazard group given in the Approved list of Biological Agents (see Part 1A of the Compendium). However, where the recipient is an attenuated derivative of an acknowledged pathogen, it may be assigned to a lower hazard group than indicated in the official list if it can be demonstrated that the strain is stably deficient in genetic material that determines virulence, or has stable mutations known to sufficiently reduce virulence. For example, derivatives of *E. coli* K12 strain have been demonstrated to be avirulent, and so do not require assignment to Hazard Group 2. Work with such strains requires only level 1 containment in the vast majority of cases (although some work with particularly harmful sequences may require higher

containment). However, wild-type *E. coli*, or strains for which evidence of attenuation is not available should be handled at a minimum of containment level 2, as for any Hazard Group 2 biological agent.

13. An increasing amount of work is undertaken using tissue in cell cultures and a considerable amount of experience has been accrued with such systems. Cell lines are often difficult to grow and require specialised defined media and growth conditions (See Annex II). Once a cell line has been immortalised by an oncogene, it may be considered as posing minimal risk of oncogenicity and may be handled appropriately.

14. Well characterised, authenticated cell lines which are known to be free from adventitious agents, such as blood-borne viruses, may be handled at ACGM level 1 so long as they do not contain hazardous inserts. Work with cell lines is generally undertaken in a Class II cabinet to protect cells from contamination, and this has the effect of further reducing any residual risk to the worker. Therefore, well characterised cell lines with a history of safe use, pose little risk to the worker or the environment. Primary cell lines which are either unauthenticated or from an uncharacterised source may pose a risk on infection and should be handled in a Class II cabinet at containment level 2. Any cells lines with endogenous pathogens, those that have been deliberately infected, or primary cells from blood, lymphoid cells, neural tissue of human or simian origin, should be handled at a containment level appropriate to the risk. Similarly, animal or plant cell lines which are either unauthenticated or from an uncharacterised source may pose a risk to the environment, and should be handled appropriately.

(b) Hazards arising directly from the inserted gene

15. This is primarily concerned with cases where the product of the inserted gene has biological properties (activities) which may give rise to harm, such as toxins, cytokines, allergens, hormones etc.

16. In cases where the insert is not being expressed, or the expressed product is produced in an inactive form, such as in an insoluble inclusion body, it is unlikely that the gene product will give rise to harm. This is often the case when human genes are expressed in *E. coli*, or other prokaryotic host systems. The proteins lack the required post-translational modifications, and are pharmacologically inactive. However, this is not always the case. Many non glycosylated cytokines, for example, expressed in *E. coli* are both soluble and biologically active.

(c) Hazards arising from the alteration of existing pathogenic traits

17. Many modifications do not involve genes whose products are inherently harmful but adverse effects may nevertheless arise as the result of exacerbation or alteration of existing pathogenic traits. This may arise as the result of the product of an inserted gene acting alongside existing pathogenic determinants. Alternatively it is possible that modification of normal genes may also alter pathogenicity. In identifying any hazards the following points should be considered (the list is not exhaustive):

- Is there an increase in infectivity or pathogenicity?
- Could any disabling mutation within the recipient be overcome due to the insertion of the foreign gene?
- Does the foreign gene encode a pathogenicity determinant from a related organism? Examples of pathogenicity determinants include bacterial toxins, invasins, integrins and surface structures such as pili, LPS and capsid.
- If the foreign gene does include a pathogenicity determinant, is it feasible that this gene could contribute to the pathogenicity of the GMM?
- Is treatment available?
- Will susceptibility to antibiotics (in relation to treatment of infection) or other forms of

therapy be affected as a consequence of the genetic modification? (most GM *E. coli* K12 strains will carry antibiotic resistance markers however, they are unlikely to cause infection, so no treatment would be required).

(d) Considerations relating to whether an inserted sequence, that does not give rise to a harmful phenotype in the recipient micro-organism, could give rise to harm as a result of natural gene transfer to another, possibly related, organism.

18. In this part of the hazard identification process the question which needs to be considered is whether the nature of the inserted gene is such that it's widespread dissemination in the environment would present particular environmental concerns e.g. a drug resistance gene or an intact provirus. If so, it is important to consider whether the recipient organism would survive in the environment in the event of a breach of containment and whether the vector is mobilisable. There is little data available on plasmid transfer from disabled *E. coli* strains. It is known that K12 strains will survive for up to seven days in the gut, and for similar lengths of time in the environment. Under conditions of stress, plasmid transfer may be more likely, so it should not be assumed that transfer will not occur because a disabled strain is being used.

ii) Consideration of the likelihood that, in the event of exposure, the GMM could actually cause harm to human health.

19. The initial stages in the risk assessment process that have been outlined above involve identifying those features of the GMM which have the potential to cause harm. It is, however, recognised that in some cases, while it may be possible to draw up theoretical scenarios to suggest that a GMM may be hazardous to human health, there can sometimes be justification to say that the likelihood of these scenarios being realised is vanishingly small.

20. Factors which come into play when considering likelihood include the analysis of the probability that rare events may occur (e.g. the likelihood of gene transfer) and a judgement as to the fitness of the GMM (see para. 23).

21. Issues relating to the likelihood of harm arising will by their very nature be very difficult to handle in situations where there is no firm data on which to make a judgement. Therefore, caution must be applied when seeking to discount on the basis of likelihood those predicted properties of the GMM which have been identified in Section (i) as being potentially harmful. In general, the weighting given to information used in the consideration of likelihood should reflect the quality of the supporting data. Where the information is either anecdotal or based on a series of roughly-drawn assumptions, it may be necessary to assume the worst and act accordingly.

(a) Probabilistic considerations that relate to the likelihood of occurrence of rare events

22. In some instances it may be possible to assign a frequency - precise or approximate - to an event. A good example of this is plasmid transfer where there is published data to compare the frequency of transfer of mobilisable, mobilisation defective and non-transferable plasmids. Similarly the likelihood of recombination events is also open to quantitative analysis. In other cases, it may be possible to adopt only a semi-quantitative frequency or descriptive assessment of the probability, based on experience with other GMMs or with the particular working methods. For example, the rate of reversion of disabling mutations should be considered, particularly when a single mutation is relied on as the source of attenuation. Where the rate of reversion is high, the use of multiple mutations will reduce the rate of reversion.

(b) Ability of a GMM to establish an *in vivo* infection and the efficiency of subsequent *in vivo* propagation. Assessment of the 'fitness' of the GMM.

23. The concept of 'fitness' is difficult to define but it relates to the ability of a GMM to spread *in vivo*,or spread within the community. It is recognised that many of the modifications that are made to micro-organisms may theoretically make the GMM more hazardous, however, the modifications may also render the organism "less fit" than the wild-type strain, and therefore less likely to spread in the event of an accidental infection. It should not, however, be assumed that the organism is "less fit" than the parental strain, unless there is scientific evidence to support the claim. One example which relates to fitness is the situation where a harmful gene product is being expressed at high level in a bacterial strain. In many cases, such over-expression of a foreign gene is deleterious to the metabolism of the recipient cell and this results in the strain rapidly accumulating either mutations which remove the foreign gene or 'down' mutations to reduce its expression.

iii) The assignment of the general controls necessary to safeguard human health i.e. the allocation of a provisional containment level.

24. Having completed the considerations in parts (i) and (ii) it is necessary at this stage to assign the GMM to a biological agents hazard group. In practice, this may be achieved by comparing the properties of the GMM with those of the parental strain (is it more/less/equally hazardous?), and with other organisms that have already been classified. The assignment of a hazard group will indicate the minimum level of containment that is appropriate. This provisional level will then form a baseline for further considerations under (iv).

25. In many cases this assignment is likely to correspond to the containment level that is appropriate for the recipient strain (if it is a human pathogen). However, in some cases, where it is predicted that the GMM will be considerably more hazardous than the recipient strain (e.g. where a pathogenicity determinant has been cloned into a recipient that is only partially disabled) it may be appropriate to assign the GMM to a higher hazard group than the recipient strain.

26. Further consideration should be given to deciding whether the minimum requirements for the chosen containment level are adequate or whether some additional measures over and above the minimum need to be applied. It may be possible to identify some particular aspect of the experimental design or work procedures which can be improved in order to minimise the risk to human health and safety. For example, some projects may be assigned to containment level 2 with one or two additional measures taken from the requirements of containment level 3. Management systems may also need to be implemented or improved, e.g. increased monitoring by internal inspections and ensuring workers are adequately trained and fully aware of the potential hazards.

(iv) Consideration of the nature of the work to be undertaken

27. This stage in the risk assessment process involves a consideration of whether the work that will be undertaken involves any non-standard operations that may involve risks that are not accounted for in the general requirements for a containment level. Examples of activities which might lead to an increased level of exposure include the following:

- inoculation of animals or plants with GMMs;
- the use of equipment likely to generate aerosols e.g. sonication or mixing;
- the use of the GMM at large scale (see part 3A).

28. For example, where *in vivo* work is being undertaken, this may require the use of sharps, such as syringes, and increase the likelihood of infection. Furthermore, the chances of recombination or reversion may be enhanced

when work *in vivo* is undertaken, as compared to work *in vitro*.

29. If it is decided that any such non-standard operations are likely to generate risks that are not accounted for in the provisional containment assigned in Section (iii) additional control measures should be applied.

> For example, if a Class II cabinet were to be used for work involving aerosol-generating procedures, with a bacterium which is spread by the airborne route, particular care would have to be taken to ensure that it provided an adequate level of operator protection. It would thus have to be subjected to a more rigorous testing regime than normal. Therefore, in view of the susceptibility of the airflows around such a cabinet to outside perturbations, it would be best practice in this situation to test the cabinet using the KI discus method on a six-monthly basis. In addition, if the laboratory was equipped with a general ventilation system that was mechanically driven this would have to be designed so that it provided an inward airflow (negative pressure).

Risk assessment for environmental harm

(v) The identification of any hazards to the environment and the assignment of any additional containment measures.

30. The objective of an environmental risk assessment is to determine the probability of adverse consequences, or "harm", to the environment arising as a result of the escape of organisms from containment (e.g. the laboratory, room, facility, glasshouse or growth room). This assessment should include all possible routes of escape to the environment by means of waste streams/waste disposal etc.

"Harm" to the environment

31. It is difficult to set out absolute criteria for harm (see introductory guidance in Part 2). However, as an example, harm would be caused if populations of micro-organisms in one or more ecosystem were adversely affected, in terms of numbers of organisms and/or in terms of the functions of organism(s) in those ecosystems. However, in other cases, such as endangered animals like ospreys and eagles, harm could be caused if the health of an individual organism were to be adversely affected. Advice on such endangered animals or plants can be obtained from the Directorate of Rural Affairs, DOE[2] Therefore, the threshold of "harm" to marginal ecosystems or to endangered or rare species, which are easily disturbed, is likely to be lower than that of established ecosystems or ubiquitous species.

Approach to assessing environmental risk

32. Harm results if hazards are realised. Therefore, it is necessary first to identify hazards, then to assess the likelihood of their being realised and then to consider whether the consequences of their realisation are serious. This procedure allows the determination of whether and to what extent there are risks.

33. The procedure set out above is developed here for assessing risk to the environment. It is illustrated[3] throughout by considering three hypothetical examples of organisms in the environment which might be affected by the release of GMMs from containment:

- work with a GM pseudomonad in the context of possible effects on soil-borne micro-organisms;
- work with a GM bacterium pathogenic to the Grey Seal (*Halichoerus grypus*);
- work with a GM *Verticillium albo-atrum* which causes progressive wilt disease of hops.

34. In all cases, the procedure recommended for assessing environmental risk is:

- hazard identification;
- assessment of the likelihood of any identified hazards being manifested;

[2] Directorate of Rural Affairs 2C, DOE, Rm.902, Tollgate House, Houlton St., Bristol BS2 9DJ.

[3] It should be emphasised that these are not intended to be exhaustive case studies.

- assessment of the consequence of identified hazards being manifested;
- determination of risk of "harm" (likelihood x consequence);
- management (control) of risk.

(a) Hazard Identification

35. The hazards to be considered (Annex IV) will usually be:

- capacity to survive, establish, disseminate and/or displace other organisms;
- pathogenicity to animals and plants;
- potential for transfer of genetic material between the GMO and other organisms;
- products of gene expression, particularly if they are toxic;
- other negative effects on organisms;
- phenotypic and genetic stability.

36. Consideration should be given to whether any, all or none of these hazards are characteristics of the GMM, taking into account the biological characteristics of the recipient or host organism, the insert, and the final GMM.

37. The capacity to survive, establish and disseminate will be key:

- if an organism is not capable of surviving in the environment, as may be the case for many of the multiply disabled organisms used in containment (for example, some auxotrophic strains of asporogenic bacilli or *E. coli* K12), none of the other hazard areas are likely to come into play and (in most cases, but see below) the organism can probably be considered safe.
- alternatively, if an organism can survive, establish and perhaps disseminate in the environment, the other hazards should be considered.

38. When assessing whether an organism might survive in the environment, it should be remembered that this includes the guts of animals and all types of association with living organisms, as well as the possibility of living in soil, water or other sites.

39. However, even if an organism has very limited capacity to survive, it will be important to decide on the potential for the gene product to persist in the environment and cause harm or for the passive transfer of the gene to other organisms in which it may be expressed. This is most likely to be relevant if large numbers of GMMs are expected to escape via waste streams or other outlets. It is known that *E. coli* K12 will survive for up to 7 days in the gut and for similar lengths of time in the environment. Under conditions of stress, plasmid transfer may be more likely, so it should not be assumed that gene transfer will not occur in the environment because a disabled host is being used.

Example (i): consider the escape of a pseudomonad, isolated from soil but not disabled, which contains a promiscuous conjugative plasmid incorporating a gene expressing a bacteriocin toxic to a wide range of soil-borne bacteria (e.g. other fluorescent pseudomonads). The potential for gene transfer would constitute a hazard, as would the expression of the gene for the toxic protein.

Example (ii): consider the escape of a GM bacterial pathogen of the Grey Seal. If that GMM were unable to survive even for a short time in the environment, then the only likely environmental hazard would be transfer of the genes coding for the pathogenic traits to other, indigenous bacteria. However, if the GMM were able to survive in the environment, then the pathogenicity of the organism would clearly pose an additional hazard.

Example (iii): consider the escape of *V. albo-atrum* which is modified to express a harmless marker protein from a stable, chromosomally integrated gene. It is able to survive in the environment and as an important disease of hops this is a significant hazard. Given the harmless nature if the insert, it is unlikely that gene transfer to another organism would constitute a hazard.

(b) Assessment of likelihood

40. The next step is to estimate the likelihood (probability and frequency) of hazard(s) being manifested. A key factor in determining this is the

potential receiving environment. This includes the wider as well as the local environment in which the activity is to be carried out. Consideration should be given to any potential exposure of the living and non-living environment to the GMMs, and the magnitude and duration of such exposure.

41. Particular characteristics of the local environment that could contribute to manifestation of the hazard should be identified and assessed. Climatic, geographical and soil conditions, and the types of flora and fauna in the receiving environment are some of the important characteristics. For example, a plant pest which has a preferred habitat in the tropics might not be a pest in temperate regions where climatic characteristics are different.

42. When estimating probabilities and frequencies, consideration should include the number of organisms that might escape given the control measures indicated from the human health risk assessment. The probability of the realisation of a hazard will often be influenced by the number of organisms which might escape.

43. Likelihood should be expressed as "high", "medium", "low" or "negligible".

Example (i): in the case of the GM pseudomonad, exposure to the soil in the vicinity of the laboratory is a possibility. However, the likelihood of this may be very low, by virtue of the containment imposed for human health and safety. If there remained the possibility that the GMM might escape (which is likely in small amounts at, for example, ACGM level 1), the likelihood of gene transfer and of expression of the toxin would need to be assessed. This would be based on the information in Schedule 3 of the Regulations (though much of this will be based on approximations). In this example, the likelihood of gene transfer and expression might be "high".

Example (ii): in the case of the Grey Seal pathogen, the assessment would depend on where the work was carried out. If it were in a facility in a laboratory far removed from any water course then, since the potential receiving environment contained no access to the marine or littoral environment, the escape of the GMM at low numbers from the inland laboratory would result in a "negligible" likelihood that Grey Seals would be affected. However, if the same work were carried out at a coastal laboratory on the North Sea, such escapes would be likely to cause great concern as there might be a "high" likelihood that the target species would be affected (although, clearly, other considerations like season, rate of dilution, local seal population sensitivity etc. would also effect the level of concern).

Example (iii): in the case of work with *V. albo-atrum* the containment required to protect human health is low, only requiring a basic level of glasshouse containment. The likelihood of escape from such a facility is high, particularly as the fungus produces airborne spores. The likelihood of such escapes causing disease in the target species (hops) would depend on the geographical area in which the work was being done. If the glasshouse is in a hop-growing area, the likelihood of harm occurring would be "high", whereas in a non hop-growing area it may be "low" or "medium"; depending on the location and persistence of the spores.

(c) Assessment of consequence

44. After the likelihood of all hazards is assessed, the consequence of each hazard should be estimated. Again, the consequence will depend to a very large extent on the potential receiving environment, both locally and in the wider context. Consequences of hazards being manifested can be described as being "severe", "medium", "low" or "negligible". However, if the assessment of the potential consequence of a hazard were "negligible" (or "low"), then even if the probability of its manifestation was "high" the risk of harm would be "low" (See Table 6, Annex IV).

45. Evaluation of the magnitude of potential consequence is difficult, since inevitably a degree of judgement will be necessary. However some qualitative guidelines are provided for the three examples.

Example (i): for the GM pseudomonad, if the bacteriocin gene were expressed, the

effects of toxin production on other soil-borne micro-organisms might lead to "severe", "medium", "low" or "negligible" consequences. For example;

- Severe consequence: a major change in the numbers of one or more species leading to negative effects on the functioning of the ecosystem and/or other connected ecosystems (for example, significantly altering the turnover of biomass, or supply of nutrient to crops). It is unlikely that the changes would be easily reversible.
- Negligible consequence: no measurable change in any microbial population in the environment or in any ecosystem function. This does not preclude some fluctuation in indigenous microbial populations as long as this is within the range of that which could be expected naturally.

Example (ii): in the case of the Grey Seal pathogen, the consequence of any contact with the host species is likely to be "severe". Unlike for soil-borne micro-organisms, it is unlikely that small fluctuations in populations due to exposure would be treated as of "negligible" or even "medium" consequence.

Example (iii): *V. albo-atrum* is a highly infectious and serious pathogen of hops. Any contact with the host species is likely to be "severe" as once the crop is infected the fungus is extremely difficult to control and is likely to persist.

(d) Determination of risk

46. The level of the risk posed by each identified hazard may be evaluated using the matrix outlined in Table 6 in Annex IV. There will clearly be some degree of judgement needed to assess the individual components of the risk. Risk is thus defined as "high", "medium", "low" or "effectively zero".

Example (i): clearly, in the case of the GM pseudomonad, the key factor influencing the likelihood of manifestation of hazards will be the level of containment set during the first part of the risk assessment (for human health and safety). Thus, even if the consequence of gene transfer or expression by the GM pseudomonad were "severe", the resultant risk might be anything from "high" to "effectively zero". If containment were inadequate, the likelihood of hazards being manifested might be "high" and, using Table 6, the resultant risk "high", but if containment were wholly adequate the likelihood might be "negligible" and the resultant risk "effectively zero".

Example (ii): in the case of the Grey Seal pathogen, likelihood would be affected not just by containment but also by the location of the facility. The likelihood of access to host animals could be "high" at a coastal laboratory, but "effectively zero" at an inland laboratory. Using Table 6, the risk posed by a "severe" hazard would be "high" for a coastal laboratory and "effectively zero" for an inland one.

Example (iii): similarly, the level of risk associated with *V. albo-atrum* will be affected by the location of the work as well as the likelihood of escape. In a hop growing area the likelihood of access to host plants might be "high" and combined with a "severe" consequence this would lead to an overall risk of "high". On the other hand, a facility away from a hop-growing area might be associated with a "low" or "medium" risk

e) Management of Risk

47. Having assessed the risks as outlined in Table 6 Annex IV, the user should then re-evaluate whether the containment level assigned in Section (iii) is adequate to protect the environment. If all risks are "low" or "effectively zero", then no additional control measure are necessary. If any risk (i.e. the risk from a particular hazard) exceeds these levels then additional control measures should be implemented so as to reduce all risks to "low"/ "effectively zero".

Example (i): if containment were found to be inadequate (i.e. if the GM pseudomonad posed a risk of "medium" or above) then additional control measures should be taken. This may, for example, consist of an effective control of the numbers of viable organisms released in waste streams. It should be emphasised that it is unlikely that additional control measures over and above those applied for human health and safety would be required in this example.

Example (ii): similarly, containment applied to work with the Grey Seal pathogen would need to be increased if risk were "medium" or above.

Example (iii): the level of glasshouse containment for the work with *V. albo-atrum* would need to be increased so that the level of risk was reduced to "low" or "effectively zero". It is possible that such work may need to done in a growth room where more effective control can be applied (see Part 3B). (It should be noted that work with *V. albo-atrum* is controlled by plant health legislation and would require a plant health license and compliance with the associated conditions). It is recognised that there is a large degree of judgement required in setting the above "risk values". Specific advice on risk assessment and containment is available from the ACGM Secretariat.

vi) Classification into Group I/II.

48. Part of the risk assessment under regulation 7 is the classification of the GMM into Group I or Group II according to the criteria in Schedule 2 to the Regulations. The criteria require that Group I GMMs are unlikely to cause disease to humans, animals or plants and are unlikely to be harmful to the environment. Micro-organisms not fulfilling these criteria will be classified as Group II. For more detailed guidance users should refer to the Guide to the Genetically Modified Organisms (Contained Use) Regulations 1992, as amended in 1996.

49. Users should be aware that an organism can be a Group II GMM on environmental grounds alone, irrespective of any hazards that it may pose to humans. For example, plant pathogens may be of no risk to humans but should be classified as Group II if they are capable of causing harm to indigenous plants.

50. It is important to note that the classification of a GMM into Group I or II and the derivation of containment and control measures (sections (i) and (ii) above), though related, are separate procedures.

51.Nevertheless, ACGM level 1 containment together with the principles of good occupational safety and hygiene will generally be appropriate for Group I GMMs. If a GMM is classified into Group I and the assessed level of containment is above level 1 then both classification and the containment level should be checked to make sure that they are correct. It is quite possible that they are; assignment to a particular containment level does not determine the classification of a GMM.

Annex I

HAZARD IDENTIFICATION BASED ON ACCESS, EXPRESSION AND DAMAGE

THE "BRENNER SCHEME"

1. This scheme essentially provides a method of determining whether a bacterial cloning host, such as *E. coli*, might be made hazardous by cloning a foreign gene into it. As such it enables users to evaluate the hazard of the GMMs produced in a wide range of routine GM work. It allows an initial estimation of the appropriate containment and control measures. Values are assigned under the headings of Access, Expression and Damage (Tables 1, 2 and 3) and these values are combined to give an overall value which gives an indication of the level of hazard of the GMM. These considerations do not constitute a comprehensive risk assessment.

Access

2. The Access factor is an indication of the likelihood that a modified micro-organism, or the DNA contained within it, will be able to enter the human body and survive there. Depending on the organism being used, various routes of entry may need to be considered. The properties of the vector e.g. mobilisation functions should also be taken into account. Table 1 sets out relative values; examples of specific hosts and vectors in each of the categories are in Annex II. It should be noted that, with different host systems there may be different sites of occupation and numbers of micro-organisms present in the body. The person undertaking the assessment and the local GMSC should consider the potential routes of exposure before reaching a final decision on the

Table 1 Access factors for host/vector combinations

Vector	Especially disabled[1]	Disabled or non-colonising[2]	Pathogenic, colonising or wild type[3]
Non-mobilisable[4]	10^{-12}	10^{-9}	10^{-3}/1
Mobilisation-defective[5]	10^{-9}	10^{-6}	10^{-3}/1
Self moblilisable[6]	10^{-6}	10^{-3}	1

[1] **Especially disabled host** means one whose growth requires the addition of specific nutrients not available in humans or outside of the culture media and is sensitive to physical conditions or chemical agents present in man or the environment. This definition applies to certain specific organisms with an extended history of safe use, as well as some strains of *E. coli* K12 and cell or tissue culture systems where the vector does not have the ability to infect or transfer DNA to other cells (**Annex II**)

[2] **Disabled or non-colonising hosts** means a multiple auxotroph or other host which is unlikely to persist in the gut, lung, or survive outside of the culture media, e.g. most strains of *E. coli* K12 and other species (**Annex II**)

[3] **Pathogenic or colonising hosts** includes all other hosts. A value of 1 applies if it is pathogenic or non-pathogenic but able to colonise humans. A value of 10^{-3} is appropriate if it is wild type and capable of survival outside of culture (**Annex III**)

[4] **Non-mobilisable vectors** are Bom$^-$, (Nic$^-$), Mob$^-$ and Tra$^-$. They include *E. coli* plasmid vectors such as pUC, pAT153, pACYC184, pBR327 and pBR328 and their derivatives (**Annex II**)

[5] **Mobilisation defective vectors** are usually Bom$^+$ but Mob$^-$ and Tra$^-$. They include *E. coli* vectors such as pBR322, pBR325, RP4DI, pACYC177 and p15A and their derivatives (**Annex II**)

TABLE 2 Relative values for the Expression factor for an initial cloning experiment

Deliberate in-frame insertion of expressible DNA downstream of a strong promoter (e.g. P_L, P_R, *tac, trp, lac,* Cm) with the intention of maximising expression (e.g. vectors pDS-5, pUC8-I, pUC9-I).	1
Insertion of expressible DNA downstream of a strong promoter (see above) with no attempt to maximise expression	10^{-3}
Insertion of expressible DNA into a site of limited promoter activity (e.g. *Bla* promoter in pBR322).	10^{-6}
Insertion of expressible DNA at a site specifically engineered to prevent expression (e.g. pDOC55, pNH series)	10^{-9}
Non-expressible DNA, e.g. DNA with no foreseeable biological effect or gene containing introns which the host is unable to process.	10^{-12}

categorisation and control measures for a particular experiment.

3. The value assigned under Access should also be considered in the light of the relevant parts of Schedule 3 to the Contained Use Regulations, particularly any indigenous plasmids of the parent (host), their host range and stability and any other significant physiological traits of the host. Furthermore, the Access factor should take into account the structure and stability of the vector in the final GMM, its frequency of mobilisation and the capacity of the final GMM to colonise humans.

4. If an attenuated or disabled strain of an acknowledged pathogen is used, data supporting an alteration of the hazard group of the pathogen should be made available to the GMSC and HSE on request. Further guidance on disabled derivatives of pathogens, together with examples, can be found in Annex II or obtained from HSE.

Expression

5. Expression is a measure of the anticipated or known level of expression of the inserted DNA. A probability of 1 is appropriate when the expressing system is designed to produce at a maximum rate in that host. "Maximum rate" is difficult to define but would include all systems which produce either >10% soluble protein or >200 mg l^{-1} protein. Examples are expression from a *trp* promoter on a pUC-derived vector in *E. coli* K12 or from the SV40 early promoter with DHFR amplification in CHO cells in standard mammalian cell culture systems.

6. Table 2 gives some examples of the Expression factors that might be applied to an initial cloning experiment on the basis of the known properties of the promoters contained on the vector or insert and their likely activity in the GMM. Subsequent characterisation may indicate that expression is higher or lower than anticipated, for example, the gene fragment may contain an efficient promoter or be cloned in the absence of an attenuation system etc. In such cases, the expression factor should be altered as part of a re-examination of the risk assessment to reflect the actual, rather than the intended, level of expression.

7. Some vector systems utilise a promoter which is not recognised by normal host RNA polymerases, for example the T3, T7 or SP6 promoters. When cloning into these vectors, the expression factor should be that appropriate for the level of expression which is anticipated in the absence of the correct polymerase, i.e. Expression = 10^{-6} or 10^{-9}.

TABLE 3 Recommended values for Damage factors.

A toxic substance or pathogenic determinant that is likely to have a significant biological effect.	1
A biologically active substance which might have a deleterious effect if delivered to a target tissue, OR a biologically inactive form of a toxic substance which, if active, might have a significant biological effect.	10^{-3}
A biologically active substance which is very unlikely to have a deleterious effect or, for example where it could not approach the normal body level (e.g. less than 10% of the normal body level).	10^{-6}
A gene sequence where any biological effect is considered highly unlikely either because of the known properties of the protein or because of the high levels encountered in nature.	10^{-9}
No foreseeable biological effect (e.g. non-coding DNA sequence).	10^{-12}

Damage

8. The Damage factor is a measure of the likelihood of harm being caused to a person by exposure to a GMM. Damage should be considered independently of Access and Expression but obviously becomes of most importance when these factors allow for a significant dose of the active product to be generated within the body of the exposed person. Additional guidance for work involving potential oncogenic sequences, proviral genomes or prion protein genes is contained in Annex III.

9. The assessment of possible harm should be linked to the known or suspected biological activity and to the levels and nature of product required to elicit this activity. The Damage factor should in particular reflect the health considerations contained in Schedule 3 of the Contained Use Regulations such as the activity of the expressed protein and any toxic, allergenic or pathogenic effects caused by the organism. Attention should also be paid to proteins, especially bacterial/human fusion proteins, which might induce autoimmune disease in persons sensitised to the protein.

10. The biological activity of a product may well be dependent on the host cell system in which the product is expressed. For example, a number of proteins expressed at high levels in *E. coli* are in fact incorrectly folded and are present in insoluble, biologically inactive, inclusion bodies. Equally, the full biological activity of other molecules will be dependent on post translational modifications, glycosylation or renaturation which will only be achieved in certain host organisms, usually animal cells. A further consideration should be whether the protein is synthesised as an inactive fusion product. The person undertaking the assessment, advised by the local GMSC, should therefore consider the potential biological activity of the product in the context of how it has been expressed and what effect this will have had on its structure and activity.

11. The following discussion and examples, together with Table 3, may assist users and local GMSC's to assign realistic figures for Damage

based on existing knowledge. Whole ricin[1], when active is known to be toxic at very low concentrations and would merit a Damage factor of 1. In contrast, insoluble ricin within an inclusion body would merit a Damage factor of 10^{-3}. Ricin A chain or human insulin are harmful only at very high concentrations and would merit a Damage factor of 10^{-6}. Human globin would probably need to be administered intravenously in very large amounts before any effect became apparent and would therefore be assessed at 10^{-9}. A Damage assessment of 10^{-12} would be appropriate for a DNA sequence that is most unlikely to be harmful (e.g. mouse satellite DNA).

12. When cloning in *E. coli* the 'worst case' situation would arise if all the *E. coli* in a person's gut were replaced by recombinant organisms expressing a foreign polypeptide in an active form at a high rate. Assuming that none of these molecules are broken down in the gut and all are absorbed and delivered to a site where they have a biological effect, this maximum possible dose should be considered when biological activity, whether pharmacological or toxic, is assessed. For example it is unlikely that the Damage factor for insulin could be high even with a GMM expressing at the maximum rate, because the perturbation of the normal insulin levels would be low (< 10%).

13. The concept of a therapeutic dose may in some instances provide a useful relative measure but has to be applied with caution. For example, the dose administered to a patient to provide a therapeutic effect may also produce side effects which may be tolerable in the treatment of illness but are undesirable in a healthy person. Furthermore, the therapeutic dose of certain substances (e.g. steroids) may be many times the normal body burden. A more fundamental problem rests with the different routes of administration of the product under consideration. A therapeutic dose administered by intravenous, oral or other routes may present a different effective dose compared with the product being generated internally at a site colonised by the GMM.

14. In cases where expression of a polypeptide with high biological activity (e.g. a toxin) is sought, ACGM recommends that as an extra precaution an especially disabled or disabled host/vector system should be used.

Assignment to containment.

15. An indication of a GMM's potential to cause harm to human health is obtained by multiplying the individual values allocated under Access, Expression and Damage. The provisional ACGM containment level with respect to human health and safety for a particular project can then be assigned using Table 4. The provisional level of containment is important when considering the likelihood of harm arising (risk), as it largely determines the level of exposure to the GMM.

TABLE 4 Provsional containment levels for human health

OVERALL VALUE	CONTAINMENT LEVEL
10^{-15} or lower	1
10^{-12} or lower	2
10^{-9} or lower	3
10^{-6} or lower	3 or 4*
greater than 10^{-6}	4

*case by case, contact HSE

[1] If toxins such as ricin are being cloned, a licence is required under the Chemical Weapons Act 1996. Any users are required to inform the Secretary of State under the conditions of an Open General Licence, issued on 1 January 1997

Annex II

EXAMPLES OF HOST-VECTOR SYSTEMS AND ACCESS FACTORS

1. This Annex lists examples of disabled hosts or poorly mobilisable vectors and should be used in conjunction with Table 1 of Part 2A. Users should also feel free to consult HSE for advice on the status of host-vector systems not in this Annex, particularly where the person undertaking the risk assessment or the local GMSC is in doubt.

2. Since 1993 the "Access" factors of hosts and vectors have been considered separately within Table 1. The various categories were extended to include terms used in the original Group I classification in the 1992 Contained Use Regulations. These detailed criteria have now been replaced by the 1996 amending Regulations but the concepts of non-virulent strains and poorly mobilisable vectors remain substantially unaltered. There is also now some further clarification of the application of the host Access factor to species and strains which are wild-type but which are most unlikely to cause harm to human health

3. This Annex only intends to give the "key" host/vector systems and does not aim to list all of the derivatives. In listing key systems, this guidance allows flexibility in the assignment of Access factors to a particular host/vector system by the person undertaking the assessment or the local GMSC. In order to assist scrutiny by HSE and others, when assigning novel hosts or vectors to various categories users should indicate which host or vector it is derived from.

Host systems

4.The assignment of factors for Access in the main text depends on features of the host species and on the cloning vector. In Table 1, hosts are divided into three classes; "especially disabled", "disabled or non-colonising" or "pathogenic, colonising or wild type" depending on their ability to colonise or infect humans and to survive outside of culture media.

5. Factors which limit colonisation, infection or survival are often termed biological barriers and can be either inherent physiological features of the organism or the result of mutations. In this sense, certain 'wild type' (i.e. not laboratory adapted or artificially mutated) hosts often have suitable biological limitations (eg inability to grow at 37°C) and could equally well be considered to fall into the 'disabled' class of hosts for the purposes of a risk assessment with respect to human health and safety.

6. As well as the examples below, users may find it helpful to consider the guidance on the Group I classification of non-virulent strains of acknowledged pathogens (see the Guide to the Regulations, 1996 edition) when determining if a host is non-pathogenic. This together with the ability to survive or colonise humans will determine whether the host may be eligible to be considered as "disabled".

i) Especially disabled hosts

7. This category of hosts has been defined by ACGM as those which are non-pathogenic, are unlikely to survive outside of culture media and have a history of safe use. This category applies to only a few species of non-pathogenic micro-organisms and to certain well defined derivatives of acknowledged pathogens. Organisms which appear to fit the criteria above but which are not listed here should be assigned to the category of "disabled" host unless a strong case can be made for their inclusion within this category.

Non-pathogenic species

Aspergillus oryzae
Bacillus subtilis[1]
Saccharomyces cerevisiae
Schizosaccharomyces pombe
Rhizobium spp. (inc. *Bradyrhizobium*)

Derivatives of pathogenic species

8. Especially disabled derivatives of bacterial pathogens such as *E. coli* are those whose growth and survival depends on the addition of nutrients not available in humans or in the environment outside of culture media and are sensitive to agents present in humans or the environment. Examples of such mutations are diaminopimelic acid requirement, thymine auxotrophy, streptomycin dependency and deoxycholate sensitivity. This definition currently applies to a limited number of strains of *E. coli* K12 only as follows;

MRC1, MRC7, MRC8, MRC9, X1776 & X1876.

Eukaryotic cell & tissue culture systems

9. In addition to the above species, all higher eukaryote cell and tissue culture systems (plant or animal, including mammalian) can be considered as especially disabled hosts provided that the cell line is unable to colonise the worker (i.e. not their own cells) and contains no known adventitious agents which are potentially harmful. The vector used must not be able to infect or transfer DNA to other hosts (see below). For further guidance concerning work with GM human and animal viral vectors see Part 2B of the Compendium.

ii) Disabled or non-colonising hosts.

10. This category of host is been defined as having biological limitations which mean that it is unlikely to survive in the gut, lung or elsewhere. This description is generally considered to cover laboratory adapted strains (particularly multiply auxotrophic or recombination deficient mutants) as well as other non-pathogenic hosts with negligible demonstrated or suggested capacity to persist in humans and a history of safe use (such as a plant pathogen).

11. Examples include most *E. coli* K12 multiple auxotrophs and other strains and species which are non-pathogenic to humans as listed below:

E. coli K-12 or B derivatives such as -

AG1, BW313, CES201, CPLK, C600, DH1, DH5, HB101, INV1, JM83, JM101, JM103, JM105, JM107, JM109, JM110, K808, KW251, LE392, NM554, N99, N4830, NM538, NM5329, P2392, PLK-A, PLK-F, RR1, SCS1, TB1, TG2, XS127, MC1061-P3, 71-18, BB4, CSH18, DH20, DH21, NM522, PLK-F', SRB, SURE™, XL1-Blue, Y1088, Y1089, Y1090

Yeasts

Pichia pastori

Other examples

Agrobacterium tumefaciens [2]

Erwinia species [2]

Well characterized derivatives or mutants[1] of

Bacillus brevis, B. sphaericus, B. stearothermophilus and Clostridium acetobutylicum

Corynebacterium glutamicum

Klebsiella oxytoca M5a1 or KP1.

Lactococcus lactis

Lactobacillus bulgaricus

Lactobacillus helveticus

Lactobacillus plantarum

Salmonella typhimurium; well characterised derivatives such as BRD509, BRD915, BRD917, SL3261, SL3235 & TA2657

Staphylococcus aureus 8325-4

Staphylococcus carnosus

Streptomyces spp.; well characterised strains[1] of species such as *S. coelicolor, S. lividans, S. parvulus* and *S. griseus.*

[1] Limited survival should be ensured by using well characterised strains which are either auxotrophic, asporogenic or sensitive to environmental factors.

[2] see also MAFF Plant Health licence requirements and environmental considerations.

iii) Other hosts

12. Hosts which do not fall into either of the above categories, i.e. they are not laboratory adapted mutants and/or are capable of infecting or colonising humans or persisting outside of culture media should be assigned an Access factor of 10^{-3} or 1. *E. coli* strain BL21 may fall into this category, as it is not a K12 or B strain derivative and there is little evidence as to the nature of its disablement. On this basis a value of 10^{-3} is considered appropriate. A rec⁻ derivitive of the strain is also available, and would warrant a value of 10^{-6}.

13. As a general principle, ACGM recommends users working with a wild type strain to consider using alternative, especially disabled or disabled, strains or mutants of the same species. Where a non-disabled strain is used, this should be justified in the risk assessment. In the case of organisms that are biological agents (COSHH 1994) there is a requirement to substitute for a less hazardous biological agent where reasonably practicable. However, if there are no suitable alternatives the following examples may assist in assigning suitable Access factors.

14. If a host is known to be pathogenic to humans and appears for example in the Approved List of Biological Agents[3] then it should automatically be assigned an Access factor of 1 and used at a containment level consistent with its hazard group. Examples of this would include most strains of *Salmonella enterica* or *Staphylococcus aureus*.

15. Organisms which are not generally regarded as pathogens but which are capable of colonising the human gut (e.g. *Citrobacter freundii*), respiratory tract (e.g. *Branhamella catarrhalis*) or skin (e.g. *Propionibacterium acnes*) would also generally warrant an Access factor of 1.

16. A wild type host which is non-pathogenic or unlikely to colonise humans (for example *Leuconostoc mesenteroides*, *Pseudomonas putida* or *Bacillus megaterium*) but which is relatively 'robust' and could survive outside of culture media, would probably (depending on its properties) warrant an Access factor of 10^{-3} when used with a non-mobilisable or mobilisation defective vector.

Fungi

17. Most fungi are non-pathogenic and do not colonise humans and many strains or species have a proven and extended history of safe use. However, there are some pathogenic species and certain commonly used species such as *Aspergillus fumigatus* which are allergenic or can cause infections following deep puncture wounds. There is also a large variation in the behaviour of different strains of the same species; laboratory-adapted strains can differ markedly from fresh isolates. For these reasons it is difficult to reliably assign fungal species to the above categories of disabled or wild type host. The following examples as a guide to suitable Access factors for fungal hosts.

18. A laboratory adapted strain of a non-pathogenic, non-allergenic fungus, for example, *Penicillium crysogenum*, *Neurospora crassa* or *Mucor* spp. used with most types of integration vector represents a minimal risk and an Access factor of 10^{-9} is appropriate.

19. A laboratory-adapted or auxotrophic strain of an allergenic or pathogenic fungus, such as *Aspergillus niger* or *A. nidulans*, used with a vector which does not contain resistance genes to antibiotics used therapeutically against that host, is of low or moderate risk and an Access factor of 10^{-6} is generally appropriate.

20. Well characterised wild type fungi with a history of safe use or with biological barriers which will not permit them to colonise or infect humans may be suitable for an Access factor of 10^{-6}. For other wild type, non-pathogenic, fungi without a history of safe use, a value of 10^{-3} would be appropriate.

[3] Categorisation of biological agents according to hazard and categories of containment. 4th edition, 1995

21. A pathogenic strain or species, for example *Aspergillus fumigatus* or *Sporothrix schenckii* would merit an Access factor of 1 and in addition, a containment level consistent with their biological agents Hazard Group.

Vector systems.

22. The Access factor also includes an assessment of the likelihood that a vector can be transferred to another organism. The assignment of an Access factor involves classifying the vector as either 'non- mobilisable', 'mobilisation defective' or 'self- mobilisable'. The notes in Table 1 are written based upon *E. coli* plasmids, but the same principles can be used to categorise other vector systems. In order to classify such a vector, information should be available concerning the likely mechanism of transfer (if any) and of any mutations or deleted regions which will reduce transfer.

23. The precise nature of such mutations will depend on the vector, i.e. whether it is based on a plasmid or on a virus. In determining the category of vector, reference should be made to the well-known examples listed below. The vectors in i) and ii) below can generally be considered to be well characterised and poorly mobilisable for the purposes of the classification into Group I in accordance with the amended Schedule 2 of the Contained Use Regulations.

i) Non-mobilisable vectors

24. These vectors are defective in one or more functions required for transfer to other hosts. For many plasmid vectors, these are loci such as Bom (basis of mobility/bacterial origin of mobility) which is sometimes synonymous with Nic as the site of the origin of transfer (*oriT*); Mob (mobility) which supply a trans-acting peptide which interacts with Bom to promote mobilisation and Tra (transfer) genes which encode the various pili proteins and other DNA processing proteins essential for conjugation.

i) Non-mobilisable bacterial plasmid vectors

25. For *E. coli* plasmid vectors they should be Bom$^-$/(Nic$^-$), Mob$^-$ and Tra$^-$. Such vectors include:

pAT153, pACYC184, pBR327, pBR328, pUC series, pBluescript II, pMTL20, pBS, pGEM, pGEMEX, pGEM Zf, pBS, pUR222, pUCBM, pSP64, pEX series, pCAT series, pT3/T7, pEUK-C1, pEUK-C2, pMAM, pDR720, pRIT2T, pRIT5, pMSG, pSP18, pSP19, pSP6/T3, pSP6/T7, pXT1, pSUß, pEMBL18, pEMBL19, pSELECT.

Cosmid vectors - pHC79, pWE15, 16, Super Cos 1, pAA113, pAA113-X, pAA113-M

26. The following *B. subtilis* vectors can normally be considered to be non-mobilisable:

pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223 & pAB124 pBD series

27. Yeast vectors: Although yeast do not transfer genes except as part of sexual reproduction, for the purposes of risk assessment, the following vector systems in a standard yeast strain can be considered to be non-mobilisable:

integration vectors (e.g. YIp vectors)

autonomously replicating vectors using ars sequences (e.g. YRp, YCp, YLp, YARp, YPp, YXp, YHp or pYAC vectors),

vectors incorporating portions of the 2µm plasmid (e.g. YEp, YCp, YARp, YPp, YXp or YHp vectors).

(When the above yeast/bacteria shuttle vectors are grown in bacterial hosts, the Access factor should be based solely on the bacterial components of the system.)

Bacteriophage vectors

28. Lambda vectors: The criteria for non-mobilisable vectors are also considered to be met by λ vectors which have a restricted host range resulting from any of the following modifications:

Incorporation of one or more suppressible nonsense mutations in essential genes (eg *Sam7*)

Deletion of the phage attachment site (*att*) coupled with a defect in the repressor (*cI* gene) or operator site (eg temperature-sensitive *cI857* or cI insertion vectors such as λgt10)

Incorporation of the *nin* deletion which prevents propagation in the plasmid mode, together with a suppressible nonsense mutation in a essential gene or removal of the phage attachment site, or a defect in the repressor (*cI* gene) or operator.

29. Examples of non-mobilisable λ vectors include:

λCharon 3A, λgt10 (and derivatives such as λGEM2, 4 etc)

λgt WES, λEMBL3, 4 (and derivatives such as λGEM11, 12),

λgt11 (and derivatives such as λZAP, λDASHII, λFIX).

30. M13 vectors: The original ACGM Note 2 considered M13 MP2am4 and M13 MP73 disabled if they were used with a disabled host containing a transfer defective F plasmid (e.g. F *traD36*). This is no longer essential and any M13 vector used in a host containing a tra$^-$ F plasmid is considered non-mobilisable.

Vectors for higher eukaryotes

31. A number of vectors listed as *E. coli* vectors are shuttle vectors intended for transient or stable expression studies in animal or plant cell-lines. Examples such as pMSG, pCH110 and pXT1 are based on eukaryotic viral sequences (mouse mammary tumour virus, SV40 virus and MMLV respectively).

32. Part 2B of the ACGM Compendium contains guidance on the risk assessment of work involving eukaryotic viral vectors. It indicates that work with viral vectors which do not normally infect human cells or in which no infective virus can be produced represents minimal risk. ACGM level 1 is suitable for such work except where the expression of allergenic (or toxic) proteins may occur. Suitable containment for such work may be determined using the principles detailed Part 3A of the Compendium.

33. In assigning an Access factor, ACGM consider that work with eukaryotic cell lines which fulfil the requirements for especially disabled hosts, using vectors which are unable to form infective virus, will generally warrant an Access factor of 10^{-12}.

Integrated vectors

34. Vectors which are integrated into the host genome may also be considered non-mobilisable vectors. It is important to consider any mechanisms within the integrated vector which may enable, for example, transposition to other sites or replicons within the host. For further guidance on such vectors users should contact HSE.

ii) Mobilisation defective vectors

35. These are vectors which are defective in one or more transfer functions and which can only be mobilised by other elements which supply the missing functions.

Plasmid vectors

For *E. coli*, plasmid vectors which are Bom$^+$/(Nic$^+$) but Tra$^-$ and Mob$^-$, can be efficiently mobilised if they are co-resident with certain other plasmids. Examples are:

pBR322, pBR325, pET, pACYC177, p15A, pROK-1, pKK233-2, pKK338-1, pBTac1, pBTac-2, pBTrp2, pBTrp56, pKC-30, pKT279, pKT280, pKT287, pFB series, pNO1523, pSVL, pKSV-10, pGA482, pGA580, pNOS, pHSV-106, RP4Δ1.

36. It is especially important to exclude the possibility that a chosen host contains a self-mobilisable plasmid which may provide the defective products *in trans* and allow efficient mobilisation (see below).

iii) Self mobilisable vectors

37. These are vectors which are conjugative or can be mobilised by conjugative plasmids. It also

includes bacteriophage vectors which are capable of producing infective phage and infecting other hosts.

Plasmid vectors

38. These are vectors which are either self-transmissable or can be readily mobilised by co-resident conjugative plasmids i.e. they are $Bom^+/(Nic^+)$, Mob^+ and/or Tra^+.

Such plasmids include F, RP4, RSF1010 & ColE1.

In determining the presence of self-mobilisable vectors attention must be paid to the presence of chromosomally-integrated 'helper' plasmids or cloned genes which are intended to mobilise the vector to other cells (for example Ti-based systems in *Agrobacterium*).

39. Certain commonly used *E. coli* strains contain integrated or episomal copies of plasmid F, without the *traD36* deletion (or similar) which render it non-conjugative. The use of such hosts with plasmids which can be mobilised by F may well require an increase in the assigned Access factor.

E. coli strains containing Tra^+ F or F' plasmids include:

71-18, BB4, CSH18, DH20, DH21, NM522, PLK-F', SRB, SURETM, XL1-Blue,

Bacteriophage systems

40. Self-mobilisable bacteriophage vectors are those which do not have a limited host range due to mutation, and/or are capable of stable lysogeny. They include wild type λ and M13.

41. The scheme described in Part 2B provides a rational basis for determining the appropriate containment for a wide range of hosts and cloned genes. However, there are some additional considerations which affect work with prion protein genes and certain potentially harmful DNA sequences, which may not produce a harmful phenotype in the GMM but might be associated with a harmful or pathogenic phenotype if transferred to another cell type. For the purpose of this guidance, harmful DNA sequences are taken to be the following:

- oncogenic sequences (see below for a definition);
- eukaryotic viral genomic DNA, including potentially infectious DNA derived from RNA viruses (e.g. HIV provirus or cDNA from picornavirus).

ANNEX III

ADDITIONAL GUIDANCE ON SPECIFIC TYPES OF GM WORK

Work involving the cloning of potentially harmful DNA or prion proteins genes should be assessed with respect to the risks posed to human health and safety and the environment using the guidance in Part 2A and in this Annex.

Oncogenic sequences

42. DNA sequences are regarded as oncogenic if they are able to make cells tumourigenic or if they are able to give cells a growth advantage in culture. Additional guidance on these terms can be found in Parts 1B and 2B.

43. **The cloning of potentially oncogenic DNA into eukaryotic viral vectors is not covered in this annex.** The use of disabled eukaryotic viral vectors does reduce the potential for virus spread but such vectors could facilitate the transfer of oncogenic DNA sequences to human cells and therefore pose additional risks to human health. Users should refer to Part 2B of the Compendium for further guidance.

44. Cloning of oncogenic sequences in prokaryotic or lower eukaryotic cells often results in a GMM which does not express a harmful product. In such cases, the GMM may represent a low risk to human health and safety. However, the possibility of the oncogene being transferred to other cells where it may be harmful should be considered.

45. It is recommended that, wherever practicable, potentially harmful DNA sequences should be cloned using especially disabled or disabled hosts and poorly mobilisable vectors. Where such work involves micro-organisms which are capable of colonising humans, it may be necessary to adopt additional containment and control measures above those considered appropriate based on the phenotype of the GMM. The use of human cell lines which may colonise the worker (such as the worker's own cells) should be especially avoided.

46. As a general guide, oncogenes that induce tumorigenicity only in immortalised cell lines (e.g. in NIH 3T3) and those that give a growth advantage to cells in culture without inducing tumorigenicity, should be assigned a Damage factor (see Part 2A) of 10^{-6}. Oncogenes that immortalise primary cell lines should be assigned a factor of 10^{-3}. Combinations of oncogenes may induce tumorigenicity more effectively. Combinations that induce tumorigenicity in primary cells, when used together, should be assigned a factor of 10^{-3} even if separately they only induce tumorigenicity in cell lines.

Oncogenes in cell lines

47. Immortal cell lines that have a history of safe use may be regarded as posing minimal risk. Recently isolated cell lines or cells made immortal by the introduction of an oncogene (e.g. SV40 large T) are potentially more hazardous but once the oncogene is integrated that they may be considered as posing minimal risk of oncogenicity and they at can normally be handled safely at ACGM level 1 with the appropriate safeguards set out below.

48. Guidance on work involving naked oncogenic DNA can be found in Part 1 (legal duties under COSHH) and Part 3A (suitable containment and control measures) of the Compendium.

Cloning of eukaryotic viral genomic DNA

49. The recommendation to use disabled or especially disabled hosts is also relevant when cloning eukaryotic virus genomic DNA. The "Damage" factor for such work should be

assigned on the basis of the guidance in Part 2A and the risk assessment should also consider whether the genome contains any harmful or pathogenic determinants, taking into account the available information on the structure and function of the viral genome.

50. All work involving cloning of eukaryotic viral genomic DNA into any host system which may produce infectious viral particles should be assigned to the appropriate level of containment using the guidance in Part 2B of the ACGM Compendium and the Approved List of Biological Agents.

51. When handling naked DNA, such as from a proviral clone, it should be recognised that the DNA is potentially infective and should be handled appropriately. If there is a risk of human infection, gloves should be worn and sharps avoided where possible. If sharps are used, care should be taken to avoid needlestick injuries.

Classification into Group I or Group II

52. Guidance on the classification of GMMs containing the above potentially harmful sequences (oncogenes and viral genomes, excluding prion protein genes) can be found in the Guide to the Regulations. The results of the risk assessment for human health set out in Part I and in this Annex should be considered carefully when classifying such GMMs. It is important to ensure that there is clear evidence that the insert will not result in a phenotype likely to cause disease. Given the potential for harm if the above sequences are transferred to other species, the vector and insert should not be self-transmissible and should be poorly mobilisable.

53. Examples of such Group I GMMs might be a transformed animal cell containing the SV40 large T-antigen or a disabled *E. coli* strain containing a cloned oncogene fragment for use as a probe sequence and which lacks the harmful biological properties of the full gene. However, other cases may be more difficult to classify and users are invited to contact HSE.

54. Bacterial GMMs which contain the full length proviral genome from any Hazard Group 3 or 4 biological agent are a special case (see the guidance on the Group I/II classification in the 1996 Guide to the Regulations). Because the consequence of any transfer to humans is potentially very serious, the likelihood of transferring such sequences to laboratory staff would have to be infinitesimally low in order to be confident that the GMM does not possess a phenotype likely to cause disease. Therefore it is recommended that such constructs are classified into Group II and are included in any notification to HSE.

55. For Type B operations, there is a further requirement that the GMM is "as safe as the parental strain in the industrial setting as the parent organism OR has characteristics that limit survival and gene transfer". If the criteria cannot be fulfilled for the cloning of harmful DNA, the work should be deemed to involve Group II GMMs and notified to HSE.

Cloning prion protein genes

56. The scheme using Access, Expression and Damage is not considered to be applicable to work with human or animal prion protein (PrP) genes, which appear to be linked to a class of disease known as transmissible spongiform encephalopathies (TSE). Because of the uncertainties surrounding the interaction between PrP genes and TSE, ACGM considers that such work should continue to follow the precautionary guidance which was set out in ACGM Newsletter 13 as summarised and updated below.

57. GMMs expressing cloned PrP genes (or other TSE-related sequences) are generally considered to be Group II GMMs and therefore, all work must be notified to HSE at least 60 days before work commences. An exception may be non-pathogenic host micro-organisms containing a cloned sub-fragment (e.g. a probe) which will not, under any circumstances, result in a harmful or pathogenic phenotype.

58. All work with PrP genes should also follow the recommendations contained in the ACDP document "Precautions for work with human and animal Transmissible Spongiform Encephalopathies"[4] particularly with respect to waste treatment and decontamination.

59. When assigning the appropriate level of containment the following factors should be considered:

a) The cloning of whole or truncated PrP genes should use a host incapable of colonising humans (i.e. an Access factor of 10^{-6} or less). Details of attenuated eukaryotic viral vectors can be found in Part 2B;

b) Work involving animals should continue to follow the guidance contained in Chapter 2E. Users should also consider whether the resulting GMO fulfils the classification criteria in Schedule 2, Part II of the Regulations. The containment appropriate for such animals should be assigned on the basis of Chapter 3C, and should take into account the current ACDP guidance for animals infected with TSE (where appropriate).

TABLE 5 Recommended containment levels for work involving the cloning of human or animal prion protein genes

Expression[a] of any human PrP gene OR Expression[a] of modified[b] animal PrP gene - ACGM level 3*
Expression[a] of naturally-occurring animal PrP gene - ACGM level 2
Non-expression[c] work in disabled hosts - ACGM level 1

* The use of inwards air flow via a room ventilation system or via the use of a safety cabinet is not considered essential.

a. "Expression" is taken to mean high level expression ("maximum rate") associated with certain bacterial host-vector systems or Baculovirus-insect cell culture systems. Commonly used host-vector systems which produce lower levels of protein, such as from the *lac*ZY promoter, the SV40 promoter or certain *in vitro* promoter systems (e.g. T7) may be suitable for a lower level of containment. Such requests for down-grading should be included along with any notification to HSE.

b. "Modified" refers to prion proteins which have an altered amino acid sequence, particularly those analogous to, or identical with, polymorphisms associated with human disease. The term also applies to prion proteins which are not post-translationally processed (e.g. lacking glycosylation, glycolipids or retaining the C-terminal portion) or those which are expressed as a fusion or truncated peptide. The latter two categories of "modified" may be suitable for down-grading to a lower level of containment.

c. "Non-expression" means either insertion into a site of limited promoter activity or at a site engineered to prevent expression (Expression $=10^{-6}$ or 10^{-9} in Table 2) and includes promoters which are not recognised by normal host RNA polymerase, such as the T7 or SP6 promoters.

All work with whole or truncated PrP genes cloned into prokaryotic or eukaryotic micro-organisms should continue to be assigned to the minimum containment level set out in Table 5. Particular attention is drawn to the provision for a case-by-case review of the assigned containment level in the light of a detailed risk assessment which may support a down-grading of containment.

[4] Advisory Committee on Dangerous Pathogens, 1994, London, HMSO (ISBN 0 11 321805 2).

ANNEX IV

ENVIRONMENTAL HAZARDS OTHER THAN POTENTIAL TO SURVIVE, ESTABLISH AND DISSEMINATE

i) Pathogenicity to animals and plants

60. The characteristics of the recipient and the GMM which are relevant to pathogenicity, infectivity, toxicity, virulence, allergenicity, colonisation, predation, parasitism, symbiosis and competition etc. should be considered. If the recipient organism is invasive or pathogenic then the GMM may exhibit the same characteristic in the environment, to a greater or lesser extent.

ii) Potential for transfer of genetic material between the GMM and other organisms

61. Concerns in this area centre around the presence of conjugative plasmids, transmissible vectors or transposable elements which could contribute to the undesirable spread of genetic material between the GMM and other organisms.

iii) Products of gene expression that could be toxic to other organisms

62. A GMM which has the potential to cause negative effects on other organisms as a result of an inserted gene coding for a toxic product will pose a hazard, even if such effects are part of the intended purpose of the GMM (e.g. a biopesticide). As an example, the gene product might kill, and reduce populations of, native non-target flora and fauna; in such a case the hazard will be affected by the level of expression and activity of the gene product.

iv) Potential to cause any other identifiable negative effects on organisms (target and non-target)

63. Any other negative effects not covered above should be considered.

v) Phenotypic and genetic stability

64. The loss of a gene inserted into the GMM will likely not constitute a hazard *per se*. However, genetic instability which leads to incorporation of genes elsewhere in the genome of the same GMM may be hazardous, producing, for example, variability in expression. Genetic instability may give rise to phenotypic instability and consideration should be given to any possible detrimental effects that this could cause.

TABLE 6 Estimation of Risk*

Consequence x likelihood = risk of causing "harm":

CONSEQUENCE OF HAZARD	LIKELIHOOD OF HAZARD			
	High	Medium	Low	Negligible
Severe	High	High	Medium	Effectively Zero
Medium	High	Medium	Medium/ Low	Effectively Zero
Low	Medium/ Low	Low	Low	Effectively Zero
Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero

* This is not intended to be a definitive matrix for "risk".

PART 2B

RISK ASSESSMENT OF GENETICALLY MODIFIED HUMAN AND ANIMAL VIRUSES AND VIRAL VECTORS

Introduction

1. This part is intended to provide guidance on the risk assessment of work involving GM human and animal viruses and viral vectors. This supplements the general guidance in Parts 1 and 2 of the Compendium. For the purposes of this guidance, the term "animal" is taken in it's broadest sense, and includes both vertebrates and invertebrates.

Gene therapy

2. The use of viral vectors for gene therapy requires rigorous control of production and safety testing methods. Approval from the Medicines Control Agency[1] and the Gene Therapy Advisory Committee via the Secretariat at the Department of Health[2] should be obtained before any gene therapy research is attempted on human subjects.

Structure of the guidance

3. The following procedures for risk assessment and the assignment of containment and control measures are recommended.

(i) Consideration of the predicted properties of the genetically modified virus to determine if there are any potential mechanisms by which it could represent a hazard to human health.

(ii) Consideration of the likelihood that the genetically modified virus could actually cause harm to human health.

(iii) The assignment of the general controls necessary to safeguard human health i.e. the allocation of a provisional level of containment.

(iv) Consideration of the nature of the work to be undertaken, and assignment of additional controls if required.

(v) The identification of any hazards to the environment (particularly non-domesticated animal species) and then, on the assumption that the controls necessary to safeguard human health have already been applied, the assignment of any additional containment measures to protect the environment.

(vi) Classification into Group I/II.

4. The scheme outlined in Part 2A consisting of numerical factors under the headings of Access, Expression and Damage is not applicable for viral vectors.

(i) Consideration of the predicted properties of the genetically modified virus to determine if there are any potential mechanisms by which it could represent a hazard to human health

(a) Hazards associated with the vector

5. Particular care must be given to the assessment of vectors with an actual or potential ability to infect humans or human cells. The COSHH Regulations 1994 require that all biological agents (in this context, any virus or viral vector that may cause any infection, allergy, toxicity or any other hazards to human health) are classified into one of four hazard groups by reference to the Approved List of biological agents, or the classification criteria set out in

[1] Medicines Control Agency, Market Tower, Nine Elms Lane, London SW8 5NQ.

[2] Department of Health, Room 417, Wellington House, 133-135 Waterloo Road, London SE1 8UG Tel: 0171 972 4911

Issued: October 1997

COSHH if the virus in question does not appear in the Approved List. Further guidance can be found in Part 1A of the Compendium and in the latest edition of the Advisory Committee on Dangerous Pathogens (ACDP) publication "Categorisation of biological agents according to hazard and categories of containment."[3] Specific guidance on certain commonly used viral vectors is given in Annex III of this part of the guidance.

Viral vectors with reduced pathogenicity

6. Schedule 9, paragraph 5, of COSHH requires the prevention of exposure to a biological agent by substituting a biological agent which is less hazardous, wherever it is reasonably practicable. For genetic modification work involving viruses with a human host range, this can be equated to a statutory requirement to, whenever possible, use disabled or attenuated viral vectors with a reduced pathogenicity. Furthermore, where appropriate, use of a vector without a human host range should be considered.

7. The origin and mechanism of such attenuation should be well understood and will form an important part of the risk assessment. In assessing whether a viral vector is adequately disabled the possibility of reversion or complementation should be considered and it should be confirmed that the virus is disabled after modification. The likelihood of reversion will depend on the mechanism of attenuation; deletion mutants are less likely to revert to wild-type than point mutations or conditional lethal mutants.

8. Insertion of a gene into the site of any disabling mutation is expected to reduce the likelihood that recombination events could result in the generation of replication competent virus expressing the gene, thus increasing the effective biological containment. This principle should be followed whenever practicable, especially when working with harmful genes. Where it is proposed to insert a harmful gene into a site other than the site of a disabling mutation, full justification should be given in the risk assessment.

9. Where the viral vector is an attenuated or disabled derivative of a human pathogen it may need to be re-classified into a hazard group different from that in the Approved List of Biological Agents for the purpose of determining the appropriate provisional containment level. (Note that this should not be confused with classification into Group I or II for notification purposes - see below and further guidance in Part 1 of the ACGM Compendium.)

> As an example, wild-type adenovirus is a Hazard Group 2 biological agent but, E1a deletion derivatives of the virus cannot replicate unless the mutation is complemented *in trans*. Such a disabled vector can therefore be considered a biological agent "unlikely to cause human disease" and assigned to Hazard Group 1 with containment level 1 as the minimum level of containment. (Note that the reclassification applies only to the disabled parental virus; any harmful properties associated with the insert or the final genetically modified virus may present an increased risk and warrant additional control measures.)

10. Further specific information on disabled vectors, including adenovirus, alphavirus, baculovirus, poxvirus, herpes simplex and retrovirus vectors, is set out in Annex III. If there is any doubt about the correct biological agent hazard group for an attenuated or disabled virus, you are advised to contact HSE's Directorate of Science and Technology in Bootle.

11. Experiments using viral vectors that do not normally infect human cells in culture and for which there is no evidence of human infection are considered to represent a minimal risk to the operator and ACGM containment level 1 is sufficient to protect human health. A higher standard of containment may however be required to control risks to other species.

[3] Categorisation of biological agents according to hazard and categories of containment (4th edition, 1995 ISBN) 0-7176-1038-1)

12. Experiments which involve DNA (or RNA) plasmid vectors derived from viruses, together with cell cultures as hosts (even if the cells contain viral sequences) but in which no infectious virus particles are involved or can be produced, are covered by the guidance in Part 2A of the ACGM Compendium. Note that this does not apply to the use of packaging cell lines intended to produce mature infectious virus particles. In deciding whether infectious virus may be produced particular attention should be paid to circumstances in which an endogenous or latent virus could act as a helper sequence.

(b) Hazards arising directly from the inserted gene product

13. The insertion of additional nucleic acid sequences into a viral vector can give rise to potential adverse effects. These may result either from the direct effects of an expressed gene product or as a consequence of an alteration in the overall properties of the GMM (see section c below). In considering the direct effects particular attention should be paid to the level of expression and site of insertion of the gene(s) and whether there is a known or suspected pharmacological or physiological effect, including the possibility of effects other than those being sought in the construction.

> For example, a non-harmful human protein (such as myelin pre-protein) expressed in vaccinia virus may provoke auto-immune disease if an operator were to be accidentally infected.

14. Particular attention should be paid to the insertion of genes which may alter the growth, replication or differentiation of cells, for example; oncogenes, potentially oncogenic sequences, or genes encoding biologically active proteins (e.g. cytokines, growth factors or toxins) into viruses capable of infecting human cells. Work with such modified viruses may pose serious consequences for people who are occupationally infected or exposed. Additional containment and control measures over and above those required for the viral vector will generally be necessary and must be applied at the correct level following the risk assessment.

Oncogenes

15. ACGM recognises that there is no precise definition of an oncogene. Genes known to be involved in the generation of tumours in humans and other animals could form the basis of a definition but many other genes generate phenotypes in cultured cells that suggests they could also be involved in tumorigenesis. Such phenotypes include transformation, density independent growth, anchorage independent growth and immortalisation, terms which themselves are difficult to define.

16. Users, in consultation with their local GMSC, should pay special attention to the potential risks of work with sequences that may be regarded as oncogenic and should feel free to request further advice from HSE. The following points may assist users with their risk assessments.

17. The formation of a cancer requires the activation (by mutation or over-expression) of oncogenes and the inactivation (by mutation or deletion) of tumour suppresser genes. It is normally a multi-step process requiring the activation or inactivation of several genes, with the cells becoming progressively more tumorigenic as genetic changes accumulate. The introduction of one change into a small number of cells is unlikely to cause cancer (although it has been observed in some experimental systems). Nevertheless, particularly if the gene is stably introduced into a stem cell, that cell and its progeny may be one step nearer to forming a cancer. Such a potentially serious outcome should not be dismissed lightly.

18. Oncogenes can often induce tumorigenicity in cells in culture that already carry genetic alterations (e.g. conferring immortalisation) but it is rare for single oncogenes to induce tumorigenicity in cultured primary cells. Combinations of oncogenes can be more effective. The introduction of an adenovirus type

12 sequence that includes both the E1a and E1b genes into human retinoblasts can cause them to form tumours when inoculated into the brain (but not the skin) of immunodeficient mice. Activated *ras* does not induce tumorigenicity in primary cells from a number of different species but, in combination with a second oncogene (e.g. *myc*) can induce tumorigenicity in primary rat embryo fibroblasts. Sequences that inactivate tumour suppresser genes (e.g. dominant negative mutants, antisense constructs) may also co-operate with oncogenes.

19. Many known oncogenes and tumour suppresser genes function in cellular signalling pathways and almost any gene that encodes a protein involved in cell-to-cell or intracellular signalling, interaction with the environment, cell cycle control, differentiation or programmed cell death (apoptosis), could be regarded as potentially oncogenic in some circumstances (e.g. perhaps if expressed constitutively at high levels). For example, expression of some genes (e.g. encoding growth factors) can allow proliferation of cells which otherwise would not grow in culture (e.g. interleukin-2 expression in T lymphocytes) and expression of other genes (e.g. E6 gene of human papilloma virus type 16) can confer an extended lifespan on cells in culture which, nevertheless, still undergo senescence.

20. There is separate guidance (Part 1B of the Compendium and in Annex III to Part 2A) on handling naked oncogenic DNA and there are duties under the COSHH Regulations to assess the potential risks of such substances. There are few examples of naked DNA producing tumours in animals but in one case, activated *ras* (admittedly in a large dose and after scarification of the skin) induced transformation of mouse skin endothelial cells. Results from experiments on DNA immunisation show that gene expression can occur from injected naked DNA, so all DNA should be handled with caution, particularly where it includes harmful sequences and in situations where sharps are being used.

(c) Hazards arising from the alteration of existing pathogenic traits

21. Many modifications to eukaryotic viral vectors do not involve genes whose products are inherently harmful but adverse effects may nevertheless arise as the result of exacerbation or alteration of existing pathogenic traits. This may arise as the result of the product of an inserted gene acting alongside existing pathogenic determinants. Alternatively it is possible that modification of normal viral genes may also alter pathogenicity. In identifying any hazards associated with the modification to the virus, the following points should be considered (the list is not exhaustive):

Alteration of tissue tropism or host range: Is there a possibility that the structure of the receptor binding site will be altered or will the product of the inserted gene be incorporated on the virus surface with the possibility of forming a novel receptor binding capacity? Cell or tissue tropism may also be affected by alterations in the transcriptional control of viral genes.

Increase in infectivity or pathogenicity: Could the modified virus show an altered susceptibility to host defence mechanisms? Is the recombinant likely to have enhanced effects upon an immuno-compromised host, beyond those normally expected with the parent virus?

Recombination or complementation: Could any disabling feature or attenuation of the viral vector be overcome by recombination or complementation either following accidental infection of a laboratory worker or accidental cross-contamination of cultures in the laboratory?

Availability of prophylaxis or therapy: Will viral susceptibility to anti-viral drugs (where these are available) be affected by genetic modification? Can vaccination or normal immune status be expected to protect against the modified virus?

Deliberate alteration of tissue tropism or specificity

22. There is increasing interest in the modification of virus tropism (usually by modification of the receptor binding proteins) for

scientific or therapeutic exploitation. Given our current understanding of viral pathogenesis, the consequences of changes in tropism are difficult to predict. The techniques available for modifying tropism are in their infancy, but are likely to develop rapidly. In assessing the risk of manipulations designed to modify tropism it must be assumed that the experiments will be successful. In general, experiments designed to generate replication-competent viruses with novel tropism or other novel pathogenic characteristics will attract high levels of containment, until the biological characteristics of the recombinant have been determined. During the risk assessment of such work a number of questions need to be considered. For example,

> could the route of transmission of the modified virus be altered?
>
> what are the predicted effects of the modified virus in tissues it would not normally infect?

(ii) Consideration of the likelihood that the genetically modified virus could actually cause harm to human health

23. The initial stages in the risk assessment process that have been outlined above involve identifying those features of the GMM which have the potential to cause harm. It is, however, recognised that in some cases, while it may be possible to draw up theoretical scenarios to suggest that a modified virus may be hazardous to human health, there can sometimes be justification to say that the likelihood of these scenarios being realised is vanishingly small.

24. Factors which come into play when considering likelihood include the analysis of the probability that rare events may occur (e.g. mutations which overcome disabling mutations) and a judgement as to the fitness of the modified virus.

25. Issues relating to the likelihood of harm arising will, by their very nature, be very difficult to handle in situations where there is no firm data on which to make a judgement. Therefore, a great deal of caution must be applied when seeking to discount on the basis of likelihood those predicted properties of the modified virus which have been identified in Section (i) as being potentially harmful. In general, the weighting given to information used in the consideration of likelihood should reflect the quality of the supporting data. Where the information is either anecdotal or based on a series of roughly-drawn assumptions it may be necessary to assume the worst and act accordingly.

Probabilistic considerations that relate to the likelihood of occurrence of rare events

26. In some instances it may be possible to assign a frequency - precise or approximate - to an event. This is particularly true for in the case of recombination and reversion frequencies, leading to the production of replication competent viruses where there may be published data. In other cases, it may be possible to adopt only a semi-quantitative frequency or descriptive assessment of the probability, based on experience with this or other comparable viruses or with the particular working methods.

27. For example, the fact that co-infection of a single cell with related forms of the same virus has not been observed *in vivo* should not be taken as firm evidence of a specific partition mechanism to prevent this happening. The lack of any observed co-infection would be relevant when making a judgement on the likelihood of recombination or complementation, but such an event could not be totally discounted in the absence of firm scientific data.

28. The judgements surrounding the assessment of likelihood can be illustrated by considering a harmful gene cloned into a vector containing a single disabling mutation. If inserted at a separate site from the disabling mutation homologous recombination could produce a replication competent virus carrying the harmful gene. This could occur as a result of cross contamination with the wild-type virus in the laboratory, recombination with viral sequences in

the packaging cell line, or from infection of a person already infected with wild type virus (particularly for a virus that is present in the general population in latent or persistent form). Such events would be deemed sufficiently likely to require additional control measures. To avoid cross contamination of this type, the handling of wild-type viruses in the same laboratory as recombinant viruses, should be avoided wherever possible. Where it is not possible (due to space constraints), there should be both spatial and temporal separation of the work. This can be achieved by designating cabinets for particular types of work, or fumigating before working with wild-type after work with recombinant virus. Care should also be taken when storing or incubating cultures, with separate incubators or freezers being used, or if that is not practical, to clearly label, and store cultures on separate shelves.

29. However, if the harmful gene was inserted at the site of the disabling mutation, although it would be theoretically possible that two illegitimate recombination events could restore the replication function of the disabled virus, such an event would be considered as highly unlikely, and can be discounted in most cases.

The ability of a GM virus to establish an *in vivo* infection and the efficiency of subsequent *in vivo* propagation. Assessment of the 'fitness' of genetically modified viruses.

30. In many cases our understanding of the molecular mechanism by which a virus brings about pathogenic effects is limited. This makes it difficult to assess the effect of genetic modification upon the pathogenicity of the modified virus. However, pathogenicity is not the only factor which determines the potential of a virus to cause harm and it is equally valid to consider factors which relate to the ability of a virus to spread *in vivo* i.e. viral 'fitness'.

31. This concept of fitness is difficult to define but will clearly be important in assessing the potential for spread of virus, if there were to be a breach of containment. For example, a clearly established example of the fitness of a virus being reduced by genetic modification is the case of insertion of a foreign gene into the E3 locus of adenovirus. Such an insertion makes the virus more susceptible to immune surveillance and so the modified virus can be considered as less likely to establish an infection and spread in the community. However, it is interesting to note that in this case it can be argued that the pathogenicity of the virus is actually increased. Thus in the event of viral infection being established, for example in an immuno-compromised individual, there can be a severe inflammatory response. Another example relating to viral fitness has been demonstrated with a number of vector systems. When the insertion of a foreign gene results in a construct that is close to the packaging constraints of the virus there is a tendency for the foreign gene to be rapidly be deleted. Similarly, the loss of a gene which conferred environmental protection, for example resistance to desiccation or UV light, would also reduce the potential for spread.

32. Consideration of the fitness of a virus is a legitimate part of a risk assessment, but should not be based merely on supposition, but on established scientific knowledge. Until it can be demonstrated that a particular type of modification will render a virus less fit than the parental virus (for example by experimental data, or through the literature etc.) the precautionary principle should be followed. This is particularly so where counter-arguments can be made for the foreign insert giving the virus a competitive advantage.

(iii) Assignment of general control measures to safeguard human health

33. This stage should involve making a judgement as to the overall hazard of the recombinant virus identified from Sections (i) and (ii) above. In doing so, it should be noted that the potential to cause harm may involve a combination of the factors identified within

different subsections of the hazard identification process.

> For example, the fact that the disabling mutation in a viral vector shows a high reversion frequency may not appear to be particularly significant, if the wild-type is only weakly pathogenic. However, this high reversion frequency could have very serious consequences if the vector were to be used for the cloning of a toxin or oncogene and there would need to be some additional containment and control measures.

34. The first step in assigning control measures should be to allocate the virus as being suitable for work in one of the four broadly defined containment levels. In many cases this assignment is likely to correspond to the containment level that is appropriate for the parental virus (if it is a human pathogen). An extract from the Approved List of Biological Agents (based on a reorganisation of the Approved List) is at Annex I. This lists the viruses pathogenic to humans into Hazard Groups 2, 3 and 4. However, in some cases, where it is predicted that the modified virus will be considerably more hazardous than the parental virus (e.g. where a harmful gene has been inserted into a replication-competent virus), it may be appropriate to assign it to a higher containment level.

35. The next stage in assigning control measures should be to decide whether the minimum requirements for the chosen containment level are adequate or whether some additional measures over and above the minimum need to be applied. It may be possible to identify some particular aspect of the experimental design or work procedures which can be improved in order to minimise the risk to human health and safety. For example, some projects may be assigned to containment level 2 with one or two additional measures taken from the requirements of containment level 3. Management systems may also need to be implemented or improved e.g. increased monitoring by internal inspections and ensuring workers are adequately trained and fully aware of the potential hazards.

36. Further guidance on containment measures is provided in Part 3A or can be obtained from HSE. For Group II GMMs in Type A operations, certian paragraphs of Part 3A constitute the approved method under Regulation 12 of the Contained Use Regulations.

37. Regulation 12 of the Contained Use Regulations also sets out the underlying principles of containment and control measures for all GMM's. These include the principles of good microbiological practice and good occupational safety and hygiene. These measures are also required for work with biological agents under COSHH. In the case of GM viruses capable of infecting human cells, ACGM feels that it is prudent to also recommend the following:

- Measures should be taken to prevent cross contamination during laboratory work to minimise the possibility of adverse consequences resulting from recombination or complementation (section (c) above). It is therefore not good practice to use aliquots taken from the same bottle of medium for culturing different virus infected cell lines and laboratory workers should be discouraged from sharing bottles of medium.
- Consideration should also be given to the need for testing to detect the presence of adventitious agents and replication competent virus (RCV). It is considered to be good practice to demonstrate the absence of RCV in virus stocks, particularly where the risk assessment and adopted level of containment assumes that RCV are not present.
- In order to minimise the risk of accidental colonisation with infected cell lines, users should not infect cultures of their own cells, nor, as a general rule, those of their immediate family or other members of the laboratory.

38. The person responsible for the work should be satisfied that the laboratory local rules give effective guidance on the maintenance of laboratory discipline and on avoiding accidental inoculation. Moreover there should be a programme of internal inspections and/or active monitoring by the BSO to ensure that the local

rules are satisfactorily implemented. All workers should be trained in good laboratory techniques before commencing work and should be fully aware of the potential hazards of the work. In particular, they should have a working knowledge of the nature and importance of any disabling mutations which provide biological containment. Access to the laboratory should be limited, where appropriate, to authorised personnel and designated workers.

(iv) Consideration of the nature of the work to be undertaken.

39. This stage in the risk assessment process involves a consideration of whether the work that will be undertaken involves any non-standard operations that may involve risks that are not accounted for in the general requirements for a containment level. Examples of such activities might include the following:

- inoculation of animals with modified virus;
- the use of equipment likely to generate aerosols e.g. sonication or mixing;
- the use of high titre virus.

40. If it is decided that any such non-standard operations are likely to generate risks that are not accounted for in the provisional containment assigned in Section (iii) additional control measures should be applied.

> For example, if a Class II cabinet were to be used for work that involved aerosol generating procedures with a virus normally transmitted by the airborne route, particular care would have to be taken to ensure that it provided an adequate level of operator protection. It would thus have to be subjected to a more rigorous testing regime than normal. Therefore, in view of the susceptibility of the airflows around such a cabinet to outside perturbations, it would be best practice in this situation to test the cabinet using the KI discus method on a six-monthly basis. In addition, if the laboratory was equipped with a general ventilation system that was mechanically driven this would have to be designed so that it provided an inward airflow (negative pressure).

(v) Risk assessment for environmental protection

41.There is a requirement under the Contained Use Regulations to consider the risks to the environment. The primary consideration here is whether the virus is capable of infecting animals (vertebrates and invertebrates). Note that this guidance does not cover work involving GM plant viruses; appropriate guidance can be found in Part 2C.

42. If the virus cannot infect any species other than humans the risk assessment should include a statement to this effect together with some justification. For such cases it can be assumed that the risks to the environment will be negligible.

43. If the virus is covered by any of the Animal Health Orders (see Annex II) or if it may infect any other animals (vertebrates or invertebrates), then the assessment should consider the risks posed to the environment. Attention should also be paid to any viruses which are known to be pathogenic to wildlife (vertebrates and invertebrates) and, in particular, any endangered species which could be affected; advice on endangered species may be obtained from the DOE Directorate of Rural Affairs[4].

44. Any additional risks to the environment caused by the modification or the inserted sequences should be assessed by consideration of the following points (the list is not exhaustive):

> Survivability: is there reason to suspect that the modification carried out to the virus may result in altered survivability in the environment? Special attention should be given to effects on UV tolerance, temperature and resistance to desiccation. If the virus is capable of long term survival in the environment and there are indigenous species with which it can recombine/ re-assort, then further considerations will be the likelihood of harmful sequences being

[4] Directorate of Rural Affairs 2X, DOE, Rm 902, Tollgate House, Houlton St., Bristol BS2 9DJ

transferred to closely related viruses and the possibility that the selective pressures could lead to the emergence of mutant derivatives that are more harmful than the recombinant virus.

Alteration of tissue tropism or host range: is the modification likely to alter the tissue tropism or host range of the recombinant virus?

Increase in infectivity or pathogenicity: is the modification likely to increase the infectivity or pathogenicity of the virus vector? Is the modified virus likely to show altered susceptibility to host defence mechanisms?

Effects on other organisms: does the insert code for a protein(s) with known or suspected inhibitory, detrimental or other physiologically active effects on other organisms? Consideration should be given to possible effects other than those being sought in the construction.

Environmental release: are all potential routes of transmission or escapes to the environment known? If so, will such routes allow the modified virus and/or its products access to the organisms in which effects may be manifested?

Availability of control agents: will virus susceptibility to control agents (where these are available) be affected by genetic modification? Can vaccination (in domestic animals) or normal immune status (in any animals) be expected to protect against the modified virus?

45. Any hazards identified from these considerations should be assessed using the approach in Part 2A, Annex II of the Compendium (including Table 5), that is, by estimating the likelihood that identified hazards will be realised and the consequences. The possibility of accidental release (escape) and survival of the modified virus are important in assessing the environmental risk. If the virus is to be used at high levels of containment because of the risks to human health and safety it is likely that the control measures will also be sufficient to protect the environment.

46. There may be cases where a virus is known to have limited survivability in the environment or is known not to infect UK hosts. In such cases, the likelihood that a hazard will be realised in the environment could be considered as "low" or "effectively zero". In considering survival it is important to determine the likely route of the virus into the environment. In an aerosol, the probability of survival may be poor, but the virus may survive well in infected animal material. The ability of the virus to infect hosts and replicate within them are also important characteristics to take into account. The assessment of risks should be made with reference to Table 6 in Part 2A, which should allow a final estimate of risk to the environment to be made.

47. If the modified virus has been assigned to a low level of containment on the basis of risks to human health, and the final risk in terms of environmental safety is not considered to be "negligible", additional controls may need to be adopted and the environmental risk assessment repeated. The additional measures should seek to reduce the likelihood of environmental exposure. Particular attention will need to be given to the possible routes of escape including the disposal of infected material, in order to minimise risks of accidental spread of virus beyond the laboratory. For certain viruses the possibility of airborne spread will need to be considered, through ventilation systems or insect vectors, for example.

48. The containment level for viruses pathogenic to animals should be, as a minimum, that specified by the appropriate Agriculture department for viral pathogens controlled by Animal Health Orders. As well as notification or consent required by the Contained Use Regulations, work with such viruses may require a licence from the appropriate Agriculture Department (Annex II).

(v) Classification into Group I or II

49. The classification of GMMs into Group I or II is part of the risk assessment and determines the notification requirements for the work. The classification criteria are in Schedule 2 to the Regulations with interpretative guidance in the Guide to the Regulations (1996 edition). They

were also reproduced in ACGM Newsletter No.19. The key consideration is whether the parental organism, inserted sequences or modified virus are likely to cause disease. The risk assessment and the decisions about containment outlined above will be of value in determining the classification.

50. It can generally be assumed that any genetically modified virus that has been judged to require containment measures corresponding to 2, 3 or 4 is likely to fall within the Group II classification. There may, however, be borderline cases that are exceptions to this general rule. Similarly in some cases work that has been assigned to containment level 1 may require classification to Group II on the basis of environmental concerns.

51. Whilst the phenotype of the recombinant virus that is under construction is the primary consideration, some thought must also be given to the possibility that harmful sequences may be transferred to closely-related viruses. One important scenario which needs to be considered at this stage is the potential for a disabling mutation in the initially constructed virus to be overcome by recombination with the wild-type sequence (for example, this might happen as the result of co-infection of a cell with both the recombinant and wild-type viruses or as the result of a recombination with viral sequences present in a packaging cell line). If the repair of a single disabling mutation is reasonably foreseeable and would give rise to a virus that might be regarded as seriously harmful, then the classification should be Group II. If sequences are inserted at the site of the disabling mutation, it is reasonable to assume that the repair of the disabling mutation would result in the loss of the insert. Such a GMM may be more readily classified as Group I.

52. When considering environmental issues, if a virus is capable of persisting in the environment and exchanging genetic material with related viruses, the presence of a harmful sequence may dictate that it is classified as Group II.

53. In cases of doubt or where the vector does not meet all of the applicable criteria, the GMM should be classified as Group II and any request to consider it as a Group I GMM should be noted clearly in the notification to HSE

Annex I

EXTRACT FROM THE GUIDANCE LIST OF BIOLOGICAL AGENTS

The hazard groups of all viruses classified as biological agents are given below in this extract from the ACDP publication "Categorisation of biological agents according to hazard and categories of containment" (4th edition, 1995). Footnotes and abbreviations are given at the end of the list.

Hazard Group 4	
Arenaviridae	
Old World	
Lassa fever	
New World	
Guanarito	
Junin	
Machupo	
Sabia	
Bunyaviridae	
Nairoviruses	
Crimean/Congo haemorrhagic fever	
Filoviridae	
Ebola	
Marburg	
Reston	
Flaviviridae:	
Kyasanur forest disease	V
Omsk	V
Russian spring summer encephalitis	V
Poxviridae	
Variola[1] (major and minor)	V
Hazard Group 3	
Arenaviridae	
lymphocytic choriomeningitis	
Flexal	
Mopeia	
Bunyaviridae	
Akabane	
Germiston	
Oropouche	
Hantaviruses	
Hantaan (Korean haemorrhagic fever)	
Sin Nombre (formerly Muerto Canyon)	
Seoul	
Nairoviruses	
Bhanja	
Phleboviruses:	
Rift valley fever	V
Caliciviridae:	
Hepatitis E[2]	
Flaviviridae:	
Flaviviruses	
Dengue viruses Types 1-4	
Israel turkey meningitis	
Japanese B encephalitis	V
Murray Valley encephalitis	
Rocio	
Sal Vieja	
San Perlita	
Spondweni	
St. Louis encephalitis	
Yellow fever	V
Wesselsbron	
West Nile fever	
Tick-borne virus group	
Absettarov	V
Hanzalova	V
Hypr	V
Kumlinge	
Louping ill	V
Negishi	
Powassan	
Hepatitis C group Viruses	
Hepatitis C[2]	D
Hepadnaviridae	
Hepatitis B[2]	VD
Hepatitis D (delta)[2]	VD
Herpesviridae:	
Herpesvirus simiae (B virus)	
Poxviridae	
Monkeypox	V

Retroviridae	
Human immunodeficiency viruses[2]	
Human T-cell lymphotropic viruses (HTLV) types 1 and 2[2]	D
Simian immunodeficiency virus[3]	
Rhabdoviridae	
Rabies	V
Piry	
Togaviridae	
Alphaviruses	
Chikungunya	
Eastern equine encephalomyelitis	V
Everglades	
Getah	
Mayaro	
Middleburg	
Mucambo	
Ndumu	
Sagiyama	
Tonate	
Venezuelan equine encephalomyelitis	V
Western equine encephalomyelitis	V
Unclassified viruses	
Blood-borne hepatitis viruses not yet identified	D
Unconventional agents[4] (i) associated with:	
Creutzfeldt-Jakob disease[2]	D
Gerstmann-Sträussler-Scheinker syndrome[2]	D
Kuru[2]	D

<u>Hazard Group 2</u>

Adenoviridae	
Arenaviridae	
Amapari	
Ippy	
Latino	
Mobola	
Parana	
Pichinde	
Tamiami	
Astroviridae	
Bunyaviridae	
Bunyamwera	
California encephalitis	
Hantaviruses	
Prospect Hill	
Puumala	
Other Hantaviruses	
Nairoviruses	
Hazara	
Phleboviruses	
Sandfly fever	
Toscana	
Uukuviruses	
Other bunyaviridae known to be pathogenic	
Caliciviridae	
Norwalk	
Other Caliciviridae	
Coronaviridae	
Flaviviridae	
Other Flaviviruses known to be pathogenic	
Herpesviridae	
Cytomegalovirus	
Epstein-Barr virus	
Herpes simplex types 1 and 2	
Herpesvirus varicella-zoster	
Human B-cell lymphotropic virus 6 (HHV6)	
Human B-cell lymphyotropic virus 7 (HHV7)	
Orthomyxoviridae	
Influenza types A, B and C	V
Tick-borne orthomyxoviridae	
Dhori and Thogoto	
Papovaviridae	
BK and JC viruses	D
Human papillomaviruses	D
Paramyxoviridae	
Measles	V
Mumps	V
Newcastle disease	
Parainfluenza (Types 1 to 4)	
Respiratory syncytial virus	
Parvoviridae	
Human parvovirus (B19)	
Picornaviridae	
Acute haemorrhagic conjunctivitis virus (AHC)	
Coxsackieviruses	
Echoviruses	
Polioviruses	V
Rhinoviruses	V
Hepatoviruses	

hepatitis A (human enterovirus type 72)	V
Poxviridae	
Buffalopox	G
Cowpox[5]	G
Milker's nodes virus	G
Molluscum contagiosum virus	G
Orf	
Vaccinia[6]	
Yatapox (Tana & Yaba)	
Reoviridae	
Coltivirus	
Human rotaviruses	
Orbiviruses	
Reoviruses	
Rhabdoviridae	
Duvenhage	
Vesicular stomatitis	V
Togaviridae	
Alphaviruses	
Bebaru	
O'nyong-nyong	
Ross river	
Semliki forest	
Sindbis	
Other known alphaviruses	
Rubiviruses	
Rubella	V

Notes & abbrevations

[1] All strains including whitepox virus. Work with these viruses should not be carried out in the United Kingdom.

[2] Refer to the exemption certificate on page 136 of the Categorisation of biological agents.

[3] At present there is no evidence of disease in humans caused by Simian T-cell lymphotropic virus (STLV). As a precaution Containment Level 3 is recommended for work with them.

[4] [There is no evidence of infections caused by the agents responsible for bovine spongiform encephalopathy. Nevertheless, Containment Level 2 is recommended as a precaution for laboratory work.] - this is under review in light of recent evidence

[5] Including strains isolated from domestic cats and exotic species, e.g. elephants, cheetah.

[6] Including strains originally classified as rabbitpox.

The notations used in this extract have the following meaning:

D: list of workers exposed to this biological agent to be kept for 40 years following the last known exposure;
G: gloves should be used;
V: an effective vaccine is available.

Annex II

Pathogens Controlled By The Agriculture And Fisheries Departments

Pathogens Of Animals And Poultry.

The Importation of Animals Pathogens Order 1980 prohibits the importation into Great Britain from outside the European Community of any pathogen that may cause disease in agricultural animals or birds, or any material in which a pathogen might be carried, except under the authority of a licence in writing issued by the appropriate Minister and in accordance with the conditions of that licence. The 'appropriate Minister' in the application of this Order in England means the Minister of Agriculture, Fisheries and Food; in Scotland, the Secretary of State for Scotland; and in Wales, the Secretary of State for Wales.

The Specified Animal Pathogens Order 1993 prohibits any person in Great Britain from holding or introducing into animals a specified animal pathogen or carrier containing that pathogen except under the authority of a licence issued in writing by the appropriate Minister. The following extract lists the viral specified animal pathogens which are those organisms causing serious epidemic diseases of farm livestock:

Herpesviridae
- Aujeszkys disease

Iridoviridae
- African swine fever

Orthomyxoviridae
- Avian influenza (Fowl plague)

Paramyxoviridae

Morbilliviruses
- Rinderpest
- Peste des petits ruminants

Paramyxoviruses
- Newcastle disease

Picornaviridae

Enteroviruses
- Swine vesicular disease
- Teschen disease

Rhinoviruses
- Foot and mouth disease

Poxviridae
- Sheep pox, goat pox and horse pox
- Lumpy skin disease

Reoviridae

Orbiviruses
- African horse sickness
- Bluetongue

Retroviridae

Unclassified
- Equine infectious anaemia

Rhabdoviridae
- Rabies and rabies related viruses
- Vesicular stomatitis

Togaviridae

Alphaviruses
- Equine encepholomyelitis (eastern, western & venezuelan)

Arteriviruses

Pestiviruses
- Swine fever (hog cholera)

Bunyaviruses
- Rift valley fever

Calicivirus
- Viral haemorrhagic disease of Rabbits

Details of the requirements for the importation or handling of pathogens of animals or poultry may be obtained from:

Ministry of Agriculture, Fisheries and Food
Animal Health (Zoonoses) Division
Hook Rise South
Tolworth, KT6 7NF

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Pathogens of other animals

The following virus of rabbits is controlled by an Order made under the Pests Act 1954:

Poxviridae
Leporipoxviruses
Myxoma

Details may be obtained from:

MAFF Worplesdon Laboratory
Tangley Place
Worplesdon, GU8 3LQ

The following virus of rabbits is controlled by Orders made under the Animal Health Act 1981:

Caliciviridae
Viral haemorrhagic disease (Rabbit haemorrhagic disease)

Details may be obtained from:

Ministry of Agriculture, Fisheries and Food
Animal Health (Disease Control) Division,
Hook Rise South
Tolworth
Surrey KT6 7NF

Pathogens of fish

The following pathogens of fish cause diseases that are notifiable and controlled by Orders under the Diseases of Fish Acts 1937 and 1983, or under the Fish Health Regulations 1992 and 1993. The importations of fish and shellfish are controlled under this legislation to ensure consignments are free of the notifiable and listed diseases and any other serious diseases exotic to GB.

Birnaviridae
Infectious pancreatic necrosis
Rhabdoviridae
Infectious haematopoietic necrosis
Spring viraemia of carp
Viral haemorhagic septicaemia
Unclassified
Infectious salmon anaemia

Details may be obtained from: MAFF Fish Disease Laboratory
The Nothe
Weymouth, DT4 8UB

ANNEX III

GUIDANCE ON COMMONLY USED VIRAL VECTORS

Additional information is provided below on a number of commonly used viral systems. It is not intended as an exhaustive guidance on a particular system nor to provide a substitute for a thorough risk assessment, but rather to draw users attention to particular points that should be considered during the risk assessment.

ADENOVIRUSES

Considerations relating to the vector

1. Human adenovirus is a double-stranded DNA virus comprising over 40 serotypes all of which are categorised as Hazard Group 2 biological agents. They vary in their pathogenicity; some serotypes (Ad12) produce sub-clinical infections; some (Ad5) are associated with mild respiratory infections in children whilst others (Ad4 and Ad7) are associated with acute respiratory disease in adults. The normal route of infection in the respiratory tract is thought to be via aerosols. Adenovirus are also implicated in conjunctivitis and gastro-enteritis (Ad40, for example, causes infantile diarrhoea). Wild-type human adenovirus is a Hazard Group 2 biological agent and as such ACDP/ACGM containment level 2 should be adopted as a minimum.

2. Immunity to adenovirus infections is thought to be life-long following primary infection, although latent infection of tonsil and adenoid tissues is a frequent occurrence following childhood infection by Ad1, 2 or 5. The precise mechanism of latency is unknown, but free virus is only rarely detected in lymphoid tissue and is present in <1 in 10^7 cells. Reactivation of latent Ad5 can lead to serious complications in immuno-compromised individuals and it has been isolated at high frequency from patients with AIDS. In addition to the occurrence of latency it has also been shown that Ad2/5 E1a sequences can be found in respiratory epithelium samples from approximately 20% of normal healthy adults. However, these sequences are thought to exist in the form of fragments of the genome that are a remnant of previous exposure rather than as intact genomes that might be capable of reactivation.

3. Human serotypes do not normally infect other animals and there are few reports of virus replication following inoculation of experimental animals. The cotton rat, a species not indigenous to the UK, provides an animal model of infection. Adenoviruses can be divided into three major groups based on their ability to produce tumour in new-born rodents. Group A viruses (e.g. Ad12) are highly oncogenic; Group B viruses (e.g. Ad7) are weakly oncogenic and Group C viruses (e.g. Ad5) are non-oncogenic. However, all human adenoviruses, including Group C, transform rat cells in culture. Despite this, there is no evidence of any association between adenoviruses and human cancer.

4. Adenovirus binds initially to target cells through the fibre protein, however the cellular receptors have not been unambiguously identified. Subsequent cell entry involves interaction between the capsid penton proteins and integrins on the target cell; different serotypes may use different cellular receptors and integrins. Adenoviruses have efficient mechanisms for internalisation to cell cytoplasm from endosomes. Inside the cell, the early genes including the E1a and E1b transforming genes are expressed, leading to replication of the ~36 kb viral genome as a linear episome, and expression of the late genes which encode capsid proteins. Between ~100 and 10^5 viral particles may be produced per cell, in a lytic cycle lasting 24-48 hours depending on the virus. Adenoviruses have no mechanism for cytolysis and so in culture, most viral particles remain cell-associated. Sufficient particles are released

however, or otherwise gain access to infect neighbouring cells. After several rounds of replication, in confluent cell cultures visible plaques of infected cells are produced, in which the cells show typical cytopathic effects (rounding up of the cytoplasm and clumping into grape-like clusters).

Disabled adenovirus vector

5. The lytic cycle of Ad5 is divided into the early and late phases. The early genes are expressed from four regions of the genome. The two transcription units of the early region 1 (E1a and E1b) are responsible for cell transformation and tumourigenicity. Both alter transcriptional regulation during infection and transform by directly interacting with cell proteins involved in transcription and cell cycle regulation (e.g. p53 tumour suppresser protein).

6. The majority of defective Ad5 vectors have a deletion removing most of the E1 region preventing expression of E1a and E1b genes. The inverted terminal repeat (1 - 103 bp) and packaging signals (194 - 358 bp) must be retained for viability. Such viruses are unable to replicate except in complementing cell lines such as 293 (a human embryonic kidney cell line which expresses the left 11% of the Ad5 genome), and foreign genes can be inserted in place of the deleted E1 genes. Adenovirus cannot efficiently package genomes longer than ~105% of the wild type length, so a 2.9 kb deletion in E1 allows insertion of up to 4.7 - 4.9 kb. Vectors have been produced with additional deletions within the viral genome, removing sequences from the E3 or E4 regions. Deletion of the E3 region (which is non-essential for *in vitro* growth) allows inserts of up to approximately 8 kb to be cloned. Alternatively, deletions in parts of the E4 region can be made without affecting normal growth, permitting an extra 1.8 kb insertion.

7. Genetically modified viruses are usually produced by manipulation of partial viral genomes in bacterial plasmids; co-transfection of overlapping plasmids into 293 cells allows the generation of complete genomes by homologous recombination. Alternative strategies involve *in vitro* ligation of linearised plasmid DNA to generate the full length genome, prior to transfection of 293 cells.

8. The replication defective Ad vectors have no mechanism for long term maintenance in cells; expression in the lining of the respiratory epithelium declines with time and is limited to ~2 months. In the absence of any significant episomal replication of E1a deleted Ad5 in normal human cells, long term maintenance requires integration in to the host chromosome. This can occur at a frequency of about 1 per 10^5 pfu in exponentially growing cultures of primary human cells.

9. Replication-defective E1a vector can be considered to be unlikely to cause disease for the purposes of the Group I/II classification and any re-categorisation into a biological agent hazard group. It is, however, important to properly take account of the nature of the inserted gene and the characteristics of the final recombinant virus.

10. An E3 deletion will reduce the likelihood of a GMM causing harm as a consequence of making the virus less able to establish and maintain an infection within the cells of an infected individual i.e. the fitness of the virus as an infective agent will be reduced. It should be noted, however, that there is little evidence that the pathogenicity of an E3-deleted virus, in any cells which do become infected, will be reduced. Indeed, if anything, there may be an increased inflammatory response.

Effects of the inserted sequences

11. The risk assessment should take into consideration the likely level of expression of an inserted gene, as well as the likely biological activity of the expressed protein. The level of expression will depend both on the cell type, and the regulatory sequences used to control transcription of the inserted gene. For example,

use of the enhancer and promoter from the immediate early transcription unit of cytomegalovirus is expected to lead to high level expression in a wide variety of cell types; the promoter from a cellular "housekeeping gene" might lead to a lower level of expression in a wide variety of cells, whereas control sequences from the insulin gene might be expected to restrict expression to ß-cells of the pancreatic islets. However, it is always possible that enhancers within the adenoviral vector, in particular the E1 enhancer which overlaps with the packaging signals, could lead to a broader tissue-specificity than expected. The potential biological consequences of a certain level of expression will depend upon the protein, but a potent toxin would clearly have more potential for harm than a simple marker such as luciferase or ß-galactosidase.

Effect of genetic modification on phenotypic characteristics of adenovirus

a) Tissue tropism: Adenoviruses have the potential to infect a wide variety of cell types, although in terms of their natural pathogenicity, they may be grouped into those associated with respiratory or enteric disease. Residual "latent" virus can also be associated with lymphoid tissue. Modification to the vector (e.g. to the fibre protein gene) might alter the tissue tropism of the virus. As a result, additional tissues might become susceptible to a modified virus.

b) Altered infectivity and pathogenicity: Either the vectors themselves, or inserted genes, might have an altered infectivity of pathogenicity relative to the wild type virus. For example, Ad5 vectors with E3 deletions have increased pathogenicity at least in the cotton rat model; this may relate to the role of the E3 19K protein in inhibiting the translocation of MHC molecules to the cell surface. Deletion of E3 is expected to result in greater presentation of viral antigens to the immune system than in a normal adenoviral infection, resulting in a greater inflammatory response (which might, however, accelerate the clearance of the infected cells). Insertion of other genes, such as cytokines, might also be expected to have some influence on the interaction of the virus with the immune system.

c) Recombination and complementation. There are three or more possibilities for recombination and complementation in E1a Ad5. Homologous recombination between E1a$^-$ Ad and wild-type virus (or viral sequences in the 293 cell lines) may occur at low frequency, but in some cases the packaging limits of Ad would tend to delete the transgene or make such a recombinant unviable. It has also been shown that recombination only occurs between serotypes within a subgenus and not between subgenera. Co-replication between E1a deletants and WT could also occur due to transcomplementation. This requires co-infection of both viruses and has so far only been demonstrated at high multiplicities of infection (moi). Some cell types have been shown to complement E1a at high moi. These include HeLa cells, where the effect is thought to be due to the endogenous human papillomavirus (HPV) 18 E7 gene product in such cells. Certain other viruses, such as Epstein-Barr virus, have been shown to complement E1a mutant Ad and this possibility should be considered.

d) Vaccination and anti-viral drugs. Vaccination or anti-viral drugs are not available, although vaccines against Ad4 and 7 have been used in US military recruits. In normal healthy humans, immunity following Ad infection is thought to be life-long.

Selection of laboratory containment and control measures.

12. Adenovirus is highly infectious by the aerosol or droplet route, even when rendered replication defective. In addition, in view of its relatively robust nature, its choice as a vector for cloning harmful genes will require a rigorous approach to risk assessment. In all cases, and particularly where a potentially hazardous gene is inserted, careful attention should be paid to containment and control measures that minimise aerosol production.

Risk assessment for environmental protection

13. Adenoviruses are non-enveloped, and therefore are relatively resistant to desiccation stress and can survive in aerosols. There is no evidence that human Ad serotypes can naturally infect animals, and replication is very limited in mouse cells, for example. However, replication has been shown to occur in the lungs of experimentally infected cotton rats administered a high doses of virus.

RETROVIRUSES

Considerations relating to the vector

14. Retrovirus vectors are particularly efficient systems for the introduction of genes into dividing cells. The virion contains two copies of an RNA genome which is reverse transcribed and integrated as a DNA provirus into the chromosomal DNA of the target cell. The host range of the viruses is dependent on a number of factors including the specificity of virion envelope glycoproteins which serve as cellular receptors and on other structural proteins which influence post entry blocks to integration. Retroviruses may be subdivided as follows:

Oncoviruses not containing transforming sequences e.g. viruses of the murine (MuLV), feline (FeLV) and avian (ALV) group

15. It is from this group that most retroviral vectors have been derived. The proviruses of the simple oncoviruses contain 3 main gene groups; the *gag* gene encoding the internal structural genes, the *pol* gene encoding the reverse transcriptase and integrase functions and the *env* gene coding for the envelope glycoproteins. Transcription of the provirus is regulated by sequences in the 5' long terminal repeat (LTR) which contains enhancers and a promoter. The underlying principle of retrovirus vector systems is that sequences necessary for packing viral RNA can be identified. In all retroviruses, the major, but not necessarily the only, determinant of RNA packaging is a sequence lying between the 5' end of the RNA transcript and *gag*.

Oncoviruses containing oncogenic sequences derived by recombination with cellular sequences.

For example, Rous sarcoma virus is a replication competent virus containing the v-*src* oncogene.

Oncoviruses belonging to the Human T-Cell lymphotropic virus (HTLV) and bovine leukaemia virus (BLV) sub-group.

These viruses have complex genomes whose gene products have trans-activating and transforming properties.

Lentiviruses

A group which include immunosuppressive viruses like HIV and viruses associated with inflammatory and degenerative diseases of animals. Lentiviruses have complex genomes with some gene products possessing transactivating functions.

Spumaviruses

Others including mammary tumour virus.

Retroviruses as insertional mutagens

16. Retroviruses of the MuLV, FeLV and ALV groups can act as insertional mutagens. The principal mode of action is through transcriptional activation of genes adjacent to the site of insertion, a process which is dependent on the enhancers or the enhancer and promoter within the viral long terminal repeat. Active insertional mutagenesis of this form may be attenuated by alterations to the viral enhancer. Self inactivating (SIN) vectors have deletions in the parental U3 region containing the enhancer. The progeny SIN vector-proviruses, integrated into the target cell, lack 5' and 3' U3 regions. As such these vectors are not easily mobilisable by superinfection with wild type virus, nor are they capable of insertional mutagenesis by the process described above. A limitation of these vectors is the low titres obtained from most packaging lines. Additional guidance on the use of retrovirus vectors for producing transgenic animals is given in Part 3E.

Disabled retrovirus vectors

17. Disabled vectors have been derived from some of the above types of retrovirus. Most

replication defective systems consist of two components, a packaging cell line and the vector. The packaging cell line contains all the structural genes but has a deleted packaging sequence so that the cell line releases virus particles lacking a normal viral RNA genome. Transfection of these packaging cells with a defective retrovirus vector, containing a packaging sequence, results in incorporation of the vector RNA into virus particles. These virions may infect cells and integrate a DNA copy of the vector genome into the target cell but are incapable of further replication.

18. Transfection of a vector into a simple packaging line described above results in the generation of replication competent retrovirus (RCR) through recombination. Wild type virus can even be found in harvests made after transient transfections of this type of packaging cell line and consequently efforts have been directed at improving their safety. Second generation ones like PA317 are an improvement in that, in addition to deletion of the packaging sequence, the 3' LTR is also deleted so that two recombinations are necessary to generate a wild type virus. Nevertheless, replication competent virus can be generated in these cells.

19. Third generation packaging lines contain the packaging construct in two components, significantly reducing the frequency of recombination between vector and packaging sequences. These two component packaging lines should be used where possible.

20. It is good practice to demonstrate the absence of RCRs in vector stocks and mandatory where the level of containment proposed assumes that RCRs are not present. For most purposes direct plating of 5% of the vector stock supernatant onto mitotically active indicator cells (e.g. feline PG4 cells for amphotropic MuLV, or XC cells for ecotropic MuLV) is adequate. However, if the insert is a harmful sequence then more rigorous testing methods may be necessary. One way of ensuring that there no RCRs would be undertake three to five passages of at least 5% of the vector stock on permissive cells followed by virus detection using two independent methods. Special consideration is required when assaying for RCRs in vectors intended for use in gene therapy. In this case the assays should be conducted by a competent laboratory operating under good laboratory practice.

Containment level for retroviruses

21. For replication defective viruses the specificity of the envelope glycoproteins of the vector virus is a major consideration in assigning the appropriate level of containment. This specificity is determined by the sequences expressed within the packaging cell line. Viruses are frequently classified into ecotropic, xenotropic and amphotropic strains.

22. Ecotropic viruses replicate in the cells of the host species and sometimes in those of closely related species. Xenotropic viruses are endogenous viruses i.e, genetically acquired viruses, that may be expressed from cells of a given animal but are unable to infect cells of that species. Xenotropic viruses may infect cells of many other species with varying efficiencies.

23. Amphotropic viruses are able to infect the cells of their host and the cells of other species. Murine amphotropic viruses were originally derived from the exogenous viruses of wild mice and form the basis of the most widely used packaging cell lines that release virions capable of infecting human cells.

24. The majority of retrovirus vector systems have been based on the oncoviruses of the murine (MuLV), feline (FeLV) and avian (ALV) groups of retroviruses. These can be assigned to ACGM containment level 1 on the basis of their inherent properties. However, this minimum containment level may need to be increased, depending on the nature of the inserted genes, the type of packaging cell lines and the properties of the final virus.

25. Vectors based on complex oncoviruses like the Bovine Leukaemia Virus (BLV) group and those based on non-primate lentiviruses require special consideration as the parental viruses contain sequences with transactivating, transforming or other undesirable features.

26. Work employing GMMs or vectors based on Human T-cell lymphotropic virus (HTLV) and primate lentiviruses (all of which are Hazard Group 3 biological agents) should generally be assumed to be Group II GMMs and handled at ACGM level 3 containment, unless there is a clear justification for using a lower level of containment.

Infection of humans working with retrovirus

27. The major risks of working with retrovirus vectors are associated with needle stick injury as might occur during animal inoculation, exposure to open wounds and aerosolisation of high titre virus stocks.

Effects of the inserted sequences

28. In the discussion below it is assumed that the insert within the vector is capable of expression within target cells. In a full risk assessment, attention should be paid to the nature of the insert and the control of its expression. The definition of retrovirus vector is used in this context is the virion and not the proviral DNA used to transfect the packaging cell lines.

29. In most instances, replication defective ecotropic non-primate vectors containing an insert unlikely to be harmful in the target species can be handled at ACGM level 1 containment. Some consideration of increased containment and control measures may be needed if the product is harmful in the host species. At the very least, this should include consideration of waste disposal methods and some consideration should be given to the need for testing for the presence of replication competent virus.

30. In the case of amphotropic replication defective vectors, access to human cells is a possibility but dissemination of the vector should not occur. In most instances, an amphotropic vector containing a non-harmful insert should not need additional containment and control measures above those indicated above. Where the vector contains a sequence which may be harmful if delivered to the target tissue (e.g. Interleukin-2), it will be necessary to consider additional containment measures, ACGM level 2 should be used as a minimum. Amphotropic vectors containing functional oncogenes should be contained at ACGM level 2. In both cases, attention should be given to the possible need for testing to detect RCV.

Effects of the inserted sequences on the characteristics of the retrovirus

- Vector inserts encoding viral glycoprotein
  Consideration should be given to vectors expressing viral glycoproteins or other cell binding ligands might alter the host range of the vector. For instance the envelope glycoprotein of vesicular stomatitis virus can be incorporated into ecotropic MuLV and will widen the host range of the virions. However, only a limited number of envelope proteins will form pseudotypes in this way.
- Incorporation of endogenous retroviral elements
  Packaging cell lines may express retroviral and retroviral related sequences that can be incorporated into the virions released from the packaging line. Particular consideration should be given to this factor if new packaging lines are being developed.

Recombination and pseudotype formation

31. Attention should be paid to alterations in host range that can occur through recombination or pseudotype formation. If a packaging cell line is capable of expressing endogenous retroviral sequences or is infected by wild type virus viruses, pseudotype particles containing the vector genome within the envelope of endogenous or superinfecting virus may be produced.

32. Recombination is of particular concern in packaging cell lines and in animal experiments. Infection of a mouse with the combination of a vector and replication competent ecotropic helper virus can result in recombination with endogenous sequences. The resulting polytropic viruses have an expanded host range. Recombination between vector sequences and endogenous (genetically inherited) sequences is of the order of 10^{-2} to 10^{-4} per replication cycle where there is some sequence homology between part of the sequences.

Risk assessment for environmental protection

33. Retroviruses require close contact for their transmission and their survival in the general environment is poor. Members of the MuLV/FeLV oncovirus group frequently require high titres of virus to establish persistent infections in immunologically competent animals so that the risk of harm to the environment associated with accidental release of vectors is generally low. Special consideration should be given to the use of vectors in domestic animals or in species whose virology is not well characterised and the advice of MAFF and HSE sought if necessary. Attention should be paid to the possibility that the vector could be mobilised by a naturally occurring retrovirus in the species. For instance, an ALV vector might be assessed for work at containment level 1 in the laboratory but when introduced into birds that contain wild type ALV, level 2 animal containment might be appropriate.

34. Experiments in mice, using retrovirus vectors mixed with helper replication competent virus should be based on ecotropic vectors if possible. Because of the problems associated with retrovirus recombination in mice leading to polytropic virus production, the inoculated mice and viruses isolated from these mice should be handled at ACGM containment level 2, if the insert is a harmful sequence.

ALPHAVIRUSES

Considerations relating to the vector

35. Alphaviruses comprise several arthropod-borne viruses in the family *Togaviridae*. Alphaviruses are enzootic, naturally infecting and replicating in mosquitoes as well as other animal species including birds. Humans infected with Hazard Group 2 alphaviruses (*e.g.* Semliki Forest [SFV], Sindbis [SIN] or Ross River virus) may develop mild symptoms, but recovery is usually uncomplicated and complete. On the other hand, Venezuelan, Eastern and Western equine encephalitis virus are Hazard Group 3 viruses and may produce epidemics of encephalitis in horses or even humans with high mortality rates. Further guidance on individual alphaviruses can be found in the International Catalogue of Arboviruses[1] The following guidance is based on SFV (and the similar SIN) which are more likely to be used as vectors for genetic modification.

36. Infection of humans or animals by the aerosol route is considered unlikely unless very high concentrations of virus are used to generate the aerosols. The risk of human infection is low if the virus preparation is handled in an appropriate safety cabinet and not injected accidentally. Natural infections only occur when infected mosquitoes take a blood meal from viraemic vertebrate hosts. The risk of natural spread of these viruses (if replication occurs) from one human to another, or from an infected animal to a human, is minimal in the absence of an available competent mosquito vector. However, it is possible that blood to blood contact or infected animal bites may carry a risk of transmission. In general, adult laboratory animals are not susceptible to infection by SIN or SFV although some strains may cause encephalitis in 3 week old mice if the virus is inoculated intracerebrally.

[1] Obtainable from Dr N Karabatsos, Division for Vector-Borne Viral Diseases, Center for Infectious Disease, CDC, Fort Collins, Colorado 80522, USA.

37. The alphaviruses have a single-stranded, positive sense RNA genome which is enclosed in a capsid protein. Two glycoproteins, E1 and E2, are incorporated into the membrane that envelopes the capsid. The virus forms two polyprotein products, one translated from the viral 5' end sequence, the other from transcription of a subgenomic mRNA corresponding to the 3' third of the genome, from where the encoded proteins are processed in infected cells to their mature forms and are incorporated into the infectious virus particles.

Disabled or attenuated alphavirus vector systems

38. Disabled derivatives of SFV have been produced which ensure that the recombinant virus undergoes only one cycle of infection. *In vitro* transcription of plasmid clones generates a packaging-competent replicon RNA which also encodes the foreign gene(s) and a packaging-incompetent DI helper RNA that encodes the virus structural proteins. These RNAs are co-transfected into permissive cells to generate recombinant, infectious virus capable of only a single replicative cycle. Although the replicon RNA persists in infected cells, no virus can be produced and the extent of the infection is limited to those cells initially exposed to virus. However, replication competent virus (RCV) may be produced by recombination in the packaging cell lines (thought to be due to replicase strand switching between replicon and helper RNAs). A further modification to this system involves the use of strains with a mutation in the p62 spike protein and packaged viruses require *in vitro* treatment with chymotrypsin before they are able to infect susceptible cell lines[2].

39. Recognising that either type of disabled derivative can produce RCV, any decision to assign these disabled vectors to ACGM level 1 must be taken at a local level in consultation with the GMSC. The appropriate containment for the final modified virus will depend on the risk assessment considerations below.

40. In a further system (which is not disabled), a full length cDNA copy of the viral RNA is modified to contain a second internal subgenomic RNA promoter positioned downstream of the internal RNA promoter which expresses the structural proteins. Heterologous gene sequences are inserted immediately downstream of the second promoter. This cDNA plasmid template is transcribed *in vitro* and when the resultant RNA is transfected into susceptible cells a fully infectious alphavirus that expresses the heterologous gene(s) is produced. Recombinant infectious clones using such vectors are generally less stable, losing their inserts on repeated passage mainly due to the lack of editing function in the RNA dependent RNA polymerase.

Effects of the inserted sequences

41. It should be possible to predict, based on studies in other host-vector systems, whether or not the expression products from SFV recombinant viruses will produce pharmacological or physiological effects on vertebrates or invertebrate cells. The introduction of a known toxin gene would clearly need an assessment of the risks associated with the toxin in the context of the SFV virus recombinant as a delivery system.

Effect of genetic modification on phenotypic characteristics of alphavirus

Tissue tropism

42. If insertion of an envelope glycoprotein gene from a virus in a different family is intended the assessment should consider the possibility that the gene product might be incorporated into the virus envelope. In general, incorporation is considered to be unlikely because of incompatibility problems (including structural differences in virus envelopes, differing sites and processes involved in virus maturation). If, however, the gene was derived from a related virus then the possibility of incorporation would

[2] Berglund *et al.* (1993). Biotechnology **11**, pp916-920

be high. In assessing risks with viruses modified in such ways, it may also be necessary to consider whether or not there is evidence of pseudotype formation between the parental viruses.

Altered infectivity and pathogenicity

43. There are no known vectors for SFV in the UK and the risk of virus transmission to wildlife is considered negligible. Nevertheless, any risk assessment should consider the possibility of altered infectivity where expressed gene products may be incorporated into the virion. If a protein to be expressed is known to have an effect on the immune system this should be taken into account in the assessment.

Recombination and complementation

44. With the disabled SFV system described above, the risk of spread through humans or animals in the environment is lower than that with the full length infectious virus, since the virus can only undergo one cycle of replication. Nevertheless, there is a small risk that fully infectious virus will be generated by recombination events during RNA replication immediately following transfection and this must be considered in the overall assessment.

Vaccination and anti-viral drugs

45. No control agents or vaccines for SFV infections are available. However where the expressed proteins are derived from other viruses for which vaccination is possible, staff could be vaccinated against the original virus. For example, vaccinations are available for a number of flaviviruses including, tick-borne encephalitis (related to louping ill virus), yellow fever, and Japanese encephalitis virus.

Risk assessment for environmental protection

Route of transmission and the risk of spread of the recombinant virus

46. The recognised route of transmission of SFV virus is via infected mosquitoes as they take a blood meal from a vertebrate host. SIN and SFV were originally isolated from *Culex* and *Aedes* mosquitoes respectively but they have subsequently been shown to infect a number of other species of mosquitoes in the tropics and sub-tropics. Such arthropods are believed to be the only natural means of replicating and transmitting the viruses to susceptible vertebrates. In mosquito-free laboratories and taking into consideration the lack of known vectors for SFV in the UK, the risk of transmission to mosquitoes and vertebrates (wildlife) in the environment in the UK is considered negligible.

BACULOVIRUSES

Considerations relating to the vector

47. Baculoviruses are pathogens of a range of insects and may, in certain circumstances, pose a potential threat to such species in the natural environment. In particular, the use of baculoviruses and susceptible host organisms must be given particular attention to ensure release to the environment does not occur.

48. The most commonly used Baculovirus vector utilises the highly expressed and regulated *Autographa californica* nuclear polyhedrosis virus (AcNPV) polyhedrin promoter modified for the insertion of foreign genes. One of the major advantages of this invertebrate virus vector is the very abundant expression of recombinant proteins in cell cultures such as Sf9 from *Spodoptera frugiperda*. Note that *S. frugiperda* is classified as a plant pest and MAFF/SOAFD advice should be sought on the requirements pertaining to its use.

Disabled or attenuated baculovirus vector systems

49. Although the original virus was pathogenic for certain lepidoptera, the expression system is based on a deletion of the polyhedrin gene which renders the virus sensitive to insect larval gut conditions and to environmental factors. Polyhedrin negative baculovirus are also susceptible to desiccation and UV light; the survival time is in the order of minutes or hours (compared to days or weeks for wild-type, occluded, AcNPV baculovirus with normal polyhedrin genes). In addition, although the virus can infect insects if injected directly, its infectivity is far lower than the parental virus.

50. Baculovirus expression systems should not automatically be assigned to ACGM level 1 containment but the risk assessment should take the above into consideration before determining the appropriate containment. It has generally been accepted that baculoviruses are not capable of infecting vertebrate or plant cells and as such do not pose any inherent hazard to workers. Recently, however, there have been studies carried out which show that baculoviruses can express foreign genes under the control of mammalian specific promoters in human or rat hepatocytes[3]. However the high level of expression of recombinant proteins possible with such vectors may cause workers to be exposed to pharmacologically or physiologically active products. The potential for such exposure must be examined in the COSHH risk assessment for each place of work.

HERPES SIMPLEX VIRUS

Considerations relating to the vector

51. Herpes simplex (HSV) is a double-stranded DNA virus which occurs in two closely related serotypes, HSV-1 and HSV-2, both of which are classified Hazard Group 2 biological agents. The majority of adults are seropositive for HSV. Infection is by contact, and transmission by the aerosol route is not thought to occur. Accidental infection in the laboratory is by splash to a mucosal surface, or by entry through broken skin. Except in seronegative neonates and the immuno-compromised, HSV infection is not systemic but is limited to epithelial cells at the infection site and to the sensory ganglia that innervate that site. Very rarely (< 1 in 10^6 people/year) the virus enters the CNS and causes encephalitis. Primary infection normally results in productive infection in epithelium which probably involves millions of cells. The virus travels to the sensory ganglion where further productive infections occurs, involving perhaps hundreds to thousands of neurones. Some neurones, hundreds, become latently infected. Reactivation and recurrence of latent infection requires infection by a few latent neurones and seeding of the epithelium where millions of cells may again be productively infected.

52. Latent infection, once established, is life-long and cannot be cured. If a promoter was active during latency, lifelong expression of an inserted sequence would occur from perhaps 100-1000 cells. The precise nature of long-term gene expression during latency is uncertain. Certain constructs that incorporate the virus-specific LAT (latency associated transcript) promoter are claimed to give long-term expression in sensory neurones, but heterologous promoters that are known to function in neurones appear to be silenced in the context of the latent genome. It is apparent that long-term gene expression in latency is difficult to achieve, but where long term expression is sought, this should be a factor in assessing risk.

Disabled HSV vectors

53. Approximately half of the 70-odd genes of HSV are dispensable for growth *in vitro*. Deletion or disruption of these genes has, in many cases,

[3] Hofmann *et al.*, (1995) Proc. Natl. Acad. Sci. USA 92, 10099-10103
Boyce, F.M. and BucHer, N.L.R. (1996). Proc. Natl. Acad. Sci. USA 93, 2348-2352

been demonstrated to result in substantial attenuation in the mouse. However, careful review of the evidence supporting the attenuation will be needed if particular insertion sites are used. Insertion into, or disruption of, the following genes have been shown to cause substantial attenuation:

thymidine kinase	(gene UL23)
ribonucleotide reductase	(gene UL39, UL40)
γ34.5	(gene RL1)
IE-110 (ICPO)	(gene IE-1)
IE-4 (ICP22)	(gene US 1)
Protein Kinase	(US-3)
Glycoprotein I	(US-7)
Glycoprotein E	(US-8)

54. The gene RL1 (encoding γ34.5) and IE1 (encoding IE-110) lie in the inverted repeat (diploid) sequence flanking the long unique region of the genome. Deletion results in substantial attenuation (many logs growth reduction *in vivo*). However, recombination with a wild virus might result in a heterozygote with one normal and one deleted copy. Where an attenuating (or disabling) site and a foreign gene insert are introduced into a diploid site, the risk assessment should consider the consequences of generating a competent heterozygote by recombination with a wild-type virus.

Replication Defective Vectors

55. A number of fully disabled, replication incompetent, vectors are now available. Constructs in which the foreign gene has been inserted at the site of disablement of such vectors can be considered to provide a very high margin of safety. Corresponding helper lines are available to complement the disabling mutations and allow propagation of these vectors in the laboratory.

56. No gene has yet been identified whose function is absolutely required for the establishment and maintenance of latency. All disabled or replication-defective mutants are capable of establishing latent infection if administered at a sufficiently high dose accompanied by tissue trauma. Accidental infection would result in transient expression of a vectored gene, and in most cases, death of infected cells. However, the possibility of latency after accidental infection should be considered as part of the risk assessment.

57. If it is intended to use disabled or attenuated HSV vectors, particularly when the inserted gene encodes a protein which could potentially cause harm, the risk assessment should consider:

HSV essential genes include:

glycoprotein L (UL1)	required for production of infectious virions
Major capsid protein VP5 (UL19) Minor capsid protein VP23(UL18)	required for capsid production
glycoprotein H (UL22)	required for production of infectious virions
Major DNA binding protein (UL29)	required for DNA replication
glycoprotein B (UL27)	required for infectious virus production
Major Tegument Protein (UL48) (αTIF, Vmw65)	required for virion production
IE-175 (RSI)	transcriptional regulator required for early and late gene expression
glycoprotein D (US6)	required for infectious virion production
ICP27 (UL54)	post-transcriptional regulator

- the rate at which replication competent virus is generated;
- whether, following high dose peripheral infection, replication competent virus could be isolated from the infection site or competent or defective virus be isolated from sensory ganglia;
- the predicted latent infection rate (as measured by *in situ* hybridisation) and whether competent or defective virus could be isolated by reactivation.

Amplicons

58. 'Amplicons' are plasmids carrying an HSV origin and packaging site, and can be used as packageable vectors to deliver foreign genes. Since they carry no viral genes they are not cytotoxic and can be used as gene delivery vehicles. By definition they are helper virus dependent and stocks usually contain at least 50% helper virus. Risk assessment should consider the potential of the helper virus to complement the amplicon *in vivo* and the possibility of recombination between the amplicon and the helper. In general, the use of a disabled or attenuated helper is advisable.

Effects of the inserted sequences

59. Some approximate predictions can be made about the likely production of a gene product expressed from an efficient promoter. A primary or recurring infection of the epidermis would give transient delivery of a few micrograms; in the sensory ganglion this would be a few nanograms during primary infection and 10's of picograms during recurrence.

60. The nervous system is poorly understood and the view that nanograms or picograms of a particular protein 'would have no biological effect' either on the behaviour of the expressing cells or on the physiology of local cells is difficult to state with certainty. One exception might be 'innocuous genes" inserted at any site which disrupts a gene or transcript. Such genes would include reporter genes, like *lac*Z and CAT whose expression in all tissues in transgenic animals throughout life is known to have no pathological effect.

61. As with all viral vector systems, additional biological containment can be achieved if the cloned gene is inserted at the attenuating or disabling site. This is particularly appropriate for inserted genes with a potentially harmful phenotype.

Effects of the inserted sequences on the characteristics of HSV

Tissue tropism and host range

62. The restriction of HSV to the epithelium and sensory nerves is not due to receptor specificity since HSV is capable of binding to, and entering, a wide range of cell types. Innate defence mechanisms are thought to be of great importance in limiting the virus while specific immunity if of importance in clearing primary and recurrent infection from the epithelium.

Altered infectivity or pathogenicity

63. HSV very rarely causes an overwhelming encehalitis in apparently normal people, and the reason is unknown. The insertion of genes, into HSV, whose products are predicted to modify neuronal physiology should be contemplated with caution. The tendency for HSV to establish latent, rather than productive, infection in neurones may be due to the failure of immediate-early gene transcription in this cell type. Modifications designed to change the transcription factor requirements of the virus should be carefully risk assessed.

Recombination or complementation.

64. Recombination could occur following an accidental infection if the recipient was actively infected at the time. If the inserted gene is inserted at the attenuating or disabling site, recombination will not result in a fully competent

vector. The question of whether a pre-existing infection in an individual could provide 'helper function' to a defective or attenuated vector is more difficult to assess. It is conceivable that accidental infection with an attenuated, disabled or amplicon vector could result in complementation by a wild virus actively replicating in the victim, and it should be noted that asymptomatic virus shedding is much more common than recurrent cold sores. Recombination between two attenuated viruses, to generate a wild type virus, is readily demonstrable *in vivo* by simultaneous inoculation at a single site. Complementation of a replication defective virus by a wild-type helper is difficult to achieve *in vivo* and has been demonstrated (with low efficiency) only by simultaneous inoculation of high doses.

Availability of therapy or prophylaxis.

65. Some consideration will be needed for insertion into the commonly used TK locus because thymidine kinase negative viruses are resistant to acylovir and this therapy would be denied in the event of accidental infection. However, such viruses are still treatable using 'Foscarnet' in the unlikely event that infection with TK^- HSV should need treatment.

Risk assessment for environmental protection.

66. Humans are the only natural host for HSV. Many other species can be infected experimentally, but these appear to be "dead end" hosts. The virus is fragile, and is rapidly inactivated by dessication, lipid solvents and mild detergents. The fact that contact is required for transmission attests to the instability of the virus outside the host.

POXVIRUSES

Considerations relating to the vector

67. Vaccinia virus recombinants are useful tools for the molecular biologist and immunologist. High levels of expression can be achieved facilitating biochemical, biological and immunological characterisation of foreign genes. Several strains of vaccinia have been derived from the original vaccine material as part of the smallpox eradication campaign, common examples include Wyeth (also known as the New York Board of Health strain, NYBH), Lister and Copenhagen. Primary vaccination in humans causes a vesicular lesion at the site of inoculation usually associated with a general infection and, rarely, a viraemia between the third and tenth day. After about 7 days, the lesion crusts over and detaches, leaving a characteristic scar. Despite millions of individuals being vaccinated without effect even vaccine strains such as Lister and Wyeth can cause infections in humans. Figures from the USA in the 1960's show that out of 14 million vaccinations there were 572 hospitalisations, 9 deaths and many less severe complications. The rate of severe adverse reactions is approximately 1 in 50,000 vaccinations.

68. Vaccinia is categorised as a Hazard Group 2 biological agent in recognition that it may cause particularly severe disease in people with active skin disorders such as eczema or psoriasis or in immuno-compromised individuals such as those infected with HIV.

69. The complex and large genome of vaccinia (over 175Kb depending on the strain) contains an estimated 150-200 genes many of which are necessary to enable the virus to replicate in the cytoplasm of infected cells. Infectious progeny virus can be detected approximately 6 hours after infection and continue for about 48 hours. With the commonly used strains, the progeny virus are released by eventual cell lysis. For laboratory workers, ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin are possible routes of infection. Laboratories working with vaccinia and other poxviruses should have suitable local rules to control these potential sources of infection.

Disabled or attenuated vaccinia virus

70. Defective vaccinia strains have been produced to reduce the incidence of complications in vaccination campaigns. For example, strain LC17m8, derived from the Lister vaccine strain, is temperature sensitive and shows lower neurovirulence. Strain MVA (Modified Virus Ankara) is multiply attenuated, containing six major deletions totalling 31Kb and will not replicate in human cells. NYVAC (New York Vaccinia, derived from Copenhagen strain) is deleted for TK, haemagglutinin (HA) and over 14 other genes. Strains MVA and NYVAC are unable to produce infectious virus in humans and may be considered for use at ACGM level 1 depending on the nature of the insert.

71. The use of the thymidine kinase (TK) gene as an insertion site, creating a Tk-minus phenotype, is believed to reduce the virulence of the virus in mice but this should not be taken to imply lower virulence in man, nor to a down-grading of categorisation. Deletion of the 19kDa epidermal growth factor (EGF) homologue gene results in marked reduction in pathogenicity, although the viruses grow well in tissue culture.

Avipoxviruses

72. Fowlpox, pigeonpox and canarypox have been used as vectors for foreign genes generally with the intention to use them as vaccines. Avipoxviruses are restricted to growth in avian species. Whilst high multiplicity of infection of mammalian cells with fowlpox virus (FPV) causes cytopathic effect, there is no evidence or productive replication of the virus in those cells. Consideration of spread in the animal population should be taken into account in a risk assessment but in general for innocuous foreign gene inserts recombinants can be handled at the same category as the parent virus.

73. Attenuated derivatives of FPV (TROVAC) and canarypox virus (ALVAC) have been demonstrated to be non-virulent in a variety of immuno-suppressed animals and human volunteers. Depending on the nature of the inserted sequences, fowlpox- and canarypox-based recombinant viruses can be handled at ACGM containment level 1. Although the replication of these viruses is abortive in mammal cells, there is clear evidence that the viruses can infect mammalian cells, albeit at relatively low levels. Due consideration must therefore be given to expression of toxic gene products from inserted foreign genes.

74. There is a long, safe history of vaccination of chickens with attenuated strains of avipoxviruses. Such attenuated strains would be classified as Group I vectors. Wild-type strains, being pathogenic for birds would be classified as Group II. Attenuated vaccine strains are available from various commercial suppliers. The most extensively- studied and readily-available FPVs are attenuated derivatives of virulent strain HP-1, isolated by Mayr & Malicki in Munich (1966). Viruses passaged more than 200 times (i.e. HP1-200, HP1-220) in chick embryo fibroblasts (CEFs) are considered to be attenuated and are the basis of some commercial vaccines. They still replicate well in chick embryos and replicate moderately well in CEFs. They posses residual pathogenicity for chickens, however, resulting in systemic lesions after intravenous inoculation of lung lesions after aerosol infection. Virus passaged in CEFs more than 400 times (i.e. HP1-400, HP1-438, HP1-440) are considered to be apathogenic by all routes. These viruses replicate well in CEFs (or in the permanently, transformed quail cell line, QT-35) but poorly in chick embryos.

75. Canarypox virus has been less well studied but similar, extensively-passaged, attenuated strains have been derived by Mayr (e.g. KP1-558).

76. Other poxviruses have been used as vectors e.g.suipox and sheeppox/goatpox. Work with these viruses is regulated by MAFF (see Annex II) and should not be undertaken except in the appropriate facilities.

Effects of the inserted sequences

77. Particular attention must be given during the risk assessment to the insertion of genes that code for proteins that may have adverse physiological or pharmacological effects *in vivo*. In all known cases, poxviral infection kills the infected cell and expression of toxic products will only be an issue if they may affect other cells when released from the cell (either biosynthetically or upon death of the cell).

78. Of particular importance are recombinants intended to investigate autoimmune responses or allergenicity in animal models. In such cases, the possibility of autoimmune or allergenic reaction in persons handling the virus should be carefully assessed and consideration should be given to the use of non-replicating poxvirus vectors.

79. Lymphokine genes have been inserted into vaccinia with a view to improving the immune response and decreasing complication rates. These include IL-1, IL-2, IL-6 and γ-interferon. In some cases, such recombinants were highly attenuated with respect to the parental virus. The risk assessment should, however, consider carefully the likely effect of any other inserted proteins in determining the appropriate containment and control measures.

80. When considering the use of oncogenic inserts, as the poxviral infection is usually lethal to the cell (even to non-permissive cells) transformation of the cell is unlikely to occur.

Efffects of the inserted sequences on the characteristics of poxviruses

Tissue tropism and host range

81. Within the poxviruses, host range varies in nature and extent. Vaccinia host range includes humans and animal species such as cattle, cats, rodents, rabbits and pigs, although the virus does not appear to occur naturally in humans and has no animal reservoir. At least three host range genes can be identified in poxviruses; vaccinia contains C7L and K1L, the insertion of a third, CHO hr gene, allows the virus to grow on Chinese hamster ovary (CHO) cells. Deletion of the C7L gene and presence of CHO hr allows growth on rabbit kidney cells. None of the host range genes are receptor attachment proteins.

Altered infectivity or pathogenicity

82.The complex organisation of the poxvirus genomes means that mutations often have the overall effect of reducing infectivity or pathogenicity. There are a number of genes that are virulence factors and where modifications in expression or control may alter virulence:

Thymidine kinase	(gene J2R)
haemagglutinin	(gene A56R)
Formation of extra-cellular virus (EEV)	(gene B5R), 14K fusion (envelope antigen (F13L)
Virus growth factor	
Complement C4b binding protein	
Complement control	(C3L)
DNA ligase	(A50R)
	(N1L)
Ribonuclease reductase	(gene 14L)
Steroid dehydrogenase	(A44L)
A-type inclusion body protein ATI	(A26L)

83. In one unusual case it has been shown that the deletion of the B15R gene actually exacerbates some pathogenic properties as compared to the wild-type virus. Deletion of this gene results in an earlier onset of illness with more severe symptoms.

Recombination or complementation

84. Recombination between modified poxvirus and other poxviruses is dependent on DNA replication, co-localization of replication and DNA homology between the two viruses. With vaccinia, the likelihood of recombination *in vivo* is low because the only natural infection with related orthopoxvirus would be monkeypox which is largely restricted to Zaire. Recombination with other poxviruses, such as orf or molluscum contagiosum has not been observed *in vitro.*

Availability of therapy or prophylaxis

85. Those working with vaccinia virus should be familiar with the ACGM/ACDP guidance on vaccination[4] issued in 1990. This recommends that smallpox vaccine should not be given to those who work with vaccinia virus or related poxviruses except:

- for work with monkeypoxvirus,

- in the light of a case-by-case risk assessment. (Examples include work with modified infectious human poxvirus where there is a significantly increased hazard due to enhancement of infectivity/pathogenicity or the presence of an expressed insert; large-scale work with infectious human poxviruses, inoculation and work with animals.); and

- where the person requests it.

Further details can be found in the joint ACGM/ACDP publication.

Risk assessment for environmental protection.

86. Vaccinia virus (and other poxviruses) have the capacity to survive for considerable periods in dried material such as detached vaccination scabs. Vaccinia virus may replicate in a number of mammalian species and there are documented cases of transmission from recently vaccinated humans to wild and domesticated species. Recent experience with large scale field trials of an attenuated vaccinia-rabies vaccine in wild foxes have shown little evidence of spread to other species and the experience of the smallpox eradication campaign indicated that vaccinia is unlikely to becomes established in the wild. Nevertheless, the risk assessment should consider the possible effects on other species which could be infected following an accidental release into the environment. In particular, close attention should be paid to the disposal of infected waste material and the containment measures for any animals which may be infected with vaccinia viruses.

87. The natural history of cowpox virus has yet to be fully elucidated but it is clear that the virus has a much wider host range than vaccinia and this should be taken into account in any risk assessment. Small wild rodents are known to be carriers of poxviruses and can be carriers of cowpox virus. This may be the source of cowpox virus isolated from domestic cats.

88. The avipox host range is limited to avian species; there has only been one report of an avipoxvirus isolated from a mammal. The virus was an atypical avipoxvirus and was isolated from an already seriously ill rhinoceros in a zoo. Avipoxviruses have been isolated from a number of avian species (e.g. fowlpox, canarypox, pigeonpox, juncopox, quailpox, mynahpox). Some can infect species other than their normal hosts. There are no clear indications of pathogenic consequences (indeed pigeonpox is naturally attenuated in chickens and has been used as a vaccine) but use of attenuated avipoxvirus strains would be recommended to reduce the risk of environmental spread to other avian species.

[4] Vaccination of laboratory workers handling vaccinia and related poxviruses infectious for humans. (ACGM/ACDP joint guidance). HMSO 1990 ISBN 0 11 885450 X.

PART 3

REGULATORY REQUIREMENTS FOR DETERMINING GMO CONTAINMENT AND CONTROL MEASURES AND GENERAL GUIDANCE

Introduction

1. The Genetically Modified Organisms (Contained Use) Regulations 1992 as amended[1], require certain standards of occupational and environmental safety and containment to be met. The level of containment and control required are to be commensurate with and determined by the risk assessment.

2. The Contained Use Regulations are not the only regulations to impact on containment and control measures and users will need to take this other legislation into account.

3. The guidance in this Part is divided into two sections. The first sets out in broad terms the requirements of the Contained Use Regulations and other legislation and guidance. The second provides greater detail for specific requirements of the Contained Use Regulations which are relevant to all activities involving any GMO.

4. Subsequent Parts give more specific guidance on the selection of appropriate containment and control measures for particular types of activity and GMO:

- Part 3A - containment and control measures for laboratory and large scale activities involving GMMs;
- Part 3B - glasshouse/growth room containment conditions;
- Part 3C - containment and control measures for transgenic animals and animals infected with GMMs.

Relevant regulations and other guidance

5. The following legislation will need to be taken into account when assigning appropriate containment and control measures[2].

a) The Contained Use Regulations.

6. Activities involving genetically modified organisms (GMOs) in contained use must use barriers to limit their contact with the general population and the environment. This is achieved using physical barriers, supplemented as necessary with biological and/or chemical barriers.

7. Containment and control measures must be chosen appropriate to the risk of the activity to both human health and the environment. For work involving any GMMs there is an additional requirement to apply the principles of good microbiological practice (GMP) and good occupational safety and hygiene (GOSH). GOSH principles are detailed in paragraph 21 of this guidance. For genetically modified animals and plants these principles must be applied in so far as they are appropriate.

8. The Contained Use Regulations require that the risk assessment be used to determine the level of containment.

9. The main elements of any risk assessment for human health and environmental safety are:

- hazard identification;
- assessment of exposure;

[1] SI 1992/3217 as amended. Referred to in what follows as 'the Contained Use Regulations'.

[2] Additional guidance on this legislation may be found in Part 1 of the ACGM Compendium of guidance.

- assessment of the level of risk (by consideration of the magnitude of harmful consequences and likelihood of their being realised);
- assessment and selection of appropriate control measures (risk management), including comparison of alternative measures where necessary.
(See also the Introduction to Part 2 of the Compendium).

Approved method

10. The Contained Use Regulations also require that for Group II GMMs in Type A activities an approved method be used to determine the containment measures. The approved method is contained within Part 3A: *(whichever is the most appropriate of the following)*

- paragraphs 9 to 15 of Part 3A and the tabular summary (Table 1) for laboratory scale activities,
- paragraphs16 to 22 of Part 3A and the tabular summary (Table 2) for large scale activities.

11. These are the only parts of the compendium which are relevant to the approved method and they function as a list of measures to be drawn upon. To select and apply measures from the list at an appropriate level is a statutory duty, and not to do so is an offence.

b) The Control of Substances Hazardous to Health (COSHH) Regulations 1994[3]

12. Those GMMs which are also biological agents (i.e. micro-organisms which present a hazard to human health) must also comply with the requirements for classification, risk assessment and control measures as set out in COSHH and the associated Approved Codes of Practice (ACoPs). In particular, those biological agents in Hazard Groups 2, 3 or 4 must be handled at specified minimum containment levels. For GMMs notified under the Contained Use Regulations it will not be necessary also to notify them under COSHH.

13. It should be noted that for GMMs which are also biological agents the appropriate minimum containment level is determined by the hazard group it is assigned to under COSHH and the Approved List of biological agents. Hazard Group 2 biological agents require level 2 (or B2) as a minimum, Hazard Group 3 require level 3 (or B3) etc. (See also guidance in Part 1 of the Compendium for advice on GMMs not on the list but which are biological agents.)

14. Not all GMMs will be biological agents as defined under COSHH, but only those which present a hazard to human health. COSHH does not consider environmental risk. Containment and control measure requirements for a particular organism and in a particular activity therefore may sometimes differ between the two sets of legislation. Where there is a discrepancy the higher containment level should be applied.

15. The standards set by the containment levels described in this guidance note are equivalent to those in COSHH.

(c) Management of Health and Safety at Work Regulations (MHSWR) 1992[4]

16. There are additional duties and measures needed to comply with the MHSWR. In particular employers should appoint competent persons to assist them in complying with health and safety legislation. Although the ultimate responsibility remains with the management in the context of work with GMMs the requirement for competent persons may be satisfied by the appointment of a competent Biological Safety Officer. The MHSWR also have requirements relating to training and supervision. Part 1C of the Compendium contains a more detailed discussion of the requirements of the MHSWR.

[3] SI 1994/3246, which implements the Biological Agents Directive.

[4] S I 1992/ 2051, see also associated ACoP.

(d) The Health and Safety at Work etc. Act 1974 (HSW Act)[5]

17. Section 2(1) and 3 of the HSW Act require employers to ensure, so far as is reasonably practicable, the health, safety and welfare at work of their employees and that others who may be affected by the work are not exposed to such risks.

Discussion of the general requirements of the Contained Use Regulations and possible means of compliance

Organisational controls

Local rules

18. One of the requirements of the Contained Use Regulations is that local rules are drawn up for the safety of personnel. They should make clear the management and organisational responsibilities and duties. The local GMSC may be involved in their formulation. Items covered will depend on the local circumstances and types of work, but might include:

- selection and training of the work force (including contracted staff such as cleaners) and supervision of work;
- policy for disinfection and procedures for the disposal of potentially infective material;
- contingency plans for spillage;
- guidance for ancillary and maintenance staff, contractors and visitors;
- maintenance and test procedures for ventilation systems, high efficiency particulate air (HEPA) filters, microbiological safety cabinets (BS 5726: 1992) and other safety equipment;
- operation and maintenance of specialist equipment;
- procedures for work in particular facilities, especially at containment levels 3 and 4 (or B3 and B4), or for work with organisms which present particular hazards;
- health surveillance (where appropriate);
- systems for accident reporting;
- duties of competent person(s) such as the biological safety officer(s).

19. The HSW Act places responsibilities on employers and employees in relation to all hazards at work. The local rules therefore need to take account of other regulations and guidance.

Containment and control

20. The Contained Use Regulations set out a hierarchy of methods to determine appropriate containment measures based on the nature of the GMO and the activity:

- Group I GMMs, Type A or B activities - Good Microbiological Practice (GMP) and Good Occupational Safety and Hygiene (GOSH) principles must be applied;
- Group II GMMs, Type A activities - GMP and GOSH principles must be applied and the containment measures are to be determined by the approved method (see para 8, Part 3);
- Group II GMMs, Type B activities - GMP and GOSH principles must be applied and the appropriate level of containment chosen from B2, B3 and B4.

21. GOSH and GMP principles do not involve only physical containment, but also cover work practices and other non-physical methods of control. The GOSH principles are set out in the Regulations. They are:

- to keep workplace and environment exposure to any physical, chemical and biological agent controlled;
- to exercise engineering control methods at source and to supplement these with appropriate personal protective clothing and equipment where necessary;
- to test and maintain control measures and equipment;
- to test, when necessary, for the presence of viable process organisms outside the primary physical containment;

[5] 1974 c 37

- to provide training for personnel;
- to formulate and implement local rules for the safety of personnel.

22. The guidance contained in this Compendium takes into account these principles and gives indications of practical measures which may be used to achieve them.

(i) Selecting containment

23. For GMMs four levels of containment are recognised for both large and small scale operations. These are:

- ACGM levels 1 - 4, for small scale;
- ACGM levels B1 - B4, for large scale.

24. Similarly animal containment facilities are divided into four equivalent levels. The guidance on glasshouse/growth room containment conditions is not divided into specific containment levels but sets out a basic containment level together with a series of additional measures to control specific risks.

25. For laboratory and large scale activities involving GMMs details of the standards set for each level are given in Part 3A, [those for animal facilities in 3C]. The levels are not discrete, but are reference levels from which appropriate containment measures for a particular activity are derived. In the case of large scale processes this will normally involve consideration of unit operations.

26. The primary objective is to select physical measures and associated safety procedures appropriate to the level of risk to both human health and the environment. The risk assessment should consider the modified organism, the nature of the activity or process and the nature of any product. In some cases the level of containment and control may be dictated by the risk posed by the product or process rather than by the GMO itself.

27. Matching containment to the risk assessment recognises that activities will differ in their absolute requirements and may require adoption of items from more than one reference level. For example:

- an activity may mainly require level 2 containment, but (possibly because it is a serious plant pathogen hazardous to the local environment) the laboratory may also need an inward airflow;
- similarly an activity may need level 3 containment, but a detailed examination of the risk assessment may indicate that it is not necessary for the workplace to be sealable for fumigation.

28. This approach does not mean that facilities need to be individually tailored for each activity, nor that locally a more rigid system could not be operated. Local circumstances will need to be taken into account. The facility must be appropriate and **at least** meet the requirements identified by the risk assessment.

Environmental considerations

29. The containment measures assigned for protection of human health will not always provide appropriate protection for the environment. ACGM recommends that following the assignment of measures suitable to protect human health, an environmental risk assessment should be made and additional measures be considered and adopted where necessary. The risk assessment should be used to identify particular elements of containment which are lacking and the measures adjusted accordingly.

Animal containment

30. Where it is proposed to inoculate animals with viable GMMs, animal containment corresponding to that used in the laboratory for the micro-organisms concerned should be used (see Part 3C of the compendium). Further details of appropriate animal containment levels are also specified in the ACDP publications, "Categorisation of biological agents according to

hazard and categories of containment" and "Working safely with research animals: managment of infection risks"[6]

(ii) Measures for securing adequate control

31. The following covers mainly non-physical measures or systems of work which can be used to control workplace and environmental exposure. They offer practical ways of complying with the GOSH requirement to control exposure to all GMOs, the appropriate level of control being chosen in the light of the risk assessment. Many of the measures and systems of work may be usefully incorporated into local rules.

32. Although the measures were developed for micro-organisms many of them can also be applied to work with modified plants and animals.

33. For GMMs which are also biological agents the primary consideration should be prevention of exposure. If this is not possible, measures must be taken to control exposure appropriately. The measures below are equivalent to those set out in that part of COSHH which covers biological agents.

(a) For preventing exposure:

- eliminate the use of the GMM; for example by using gene probes and polymerase chain reaction (PCR) rather than the GMM;
- substitution with a less hazardous GMM.

(b) For controlling exposure:

- design of work processes and engineering control measures to avoid or minimise the escape of GMMs into the workplace; totally enclosed process and handling systems e.g. Class III cabinets, enclosed fermenters; partial enclosure with local exhaust ventilation e.g. microbiological safety cabinets, containment of aerosols;
- test and maintain control measures and equipment;
- provide sufficient general ventilation, which may include use of negative pressure;
- provide sufficient general ventilation, which may include use of negative pressure;
- keep as few as possible the number of workers exposed or likely to be exposed;
- prevent entry (other than in emergency) for cleaning, servicing of equipment, repairs or other activities outside the normal work of the laboratory unless a responsible member of staff has previously been informed and, in containment level (B)2 or above, laboratory surfaces have been appropriately disinfected (which may include fumigation);
- reduce the period of exposure for workers;
- provide suitable personal protective equipment;
- use hygiene measures compatible with the aim of the prevention or reduction of the accidental transfer or escape of GMMs from the workplace:

  such as

  regular cleaning of contamination from, or disinfection of surfaces, walls etc. with specified procedures;

  provision of adequate facilities for washing, changing and storage of clothing, including arrangements for laundering contaminated clothing;

  prohibition of eating, drinking, smoking, storage of food and applying cosmetics in containment areas;

  prohibition of mouth pipetting;

  providing a means for safe collection, storage, treatment and disposal of waste, including the use of secure and identifiable containers;

  arrangements for the safe handling and transport of GMOs within the workplace;

  drawing up plans to deal with accidents involving GMOs;
- test (monitor) where necessary and technically possible for the presence of the GMOs used outside the primary physical containment;
- avoid sharps except where essential for the work, use plastic pipettes or similar where the risk assessment indicates it is necessary;

[6] Published by HSE Books 1997 ISBN 0 7176 1377 1

- display a biohazard sign and other relevant warning signs;
- where appropriate, make vaccines available.

PART 3A

SELECTION OF CONTAINMENT AND CONTROL MEASURES

Introduction

1. This guidance is intended to help users determine in detail the appropriate standards of containment and control that they should apply to work involving GMMs in both laboratory and large scale activities. It should be read in conjunction with the more general guidance in Part 3 of the Compendium. It sets out four levels of containment and control for both laboratory and large scale operations (ACGM levels 1 - 4 for laboratory scale and ACGM levels B1 - B4 for large scale.)

2. Legislative requirements will be stated clearly as such. Otherwise the guidance outlines approaches which can be used to achieve the appropriate standards. These approaches are only illustrative. Users may adopt other approaches and methods so long as the standards set by the Regulations are met.

Scope

3.This guidance revises and replaces both ACGM/HSE Note 6 (1987) and ACGM/HSE Note 8 (1988). It covers all contained use work with GMMs for all purposes and at all scales, apart from glasshouse containment and animal facilities. Guidance is given on the protection of both human health and the environment.

4. Annexes contain additional advice on:

- Handling of oncogenes
- Microbiological Safety Cabinets
- Disinfection
- Fumigation

Selection of containment and control measures

5. This section details the standards of containment and control for the four levels at both small scale (Section I) and large scale (Section II). In the broadest sense measures are often similar across all levels. However, the degree of stringency and appropriateness of methods used to meet the necessary standard will differ and this is what distinguishes the levels. For instance, safe storage of GMMs is stated for all levels, although what constitutes safe storage at level 4 will be considerably more stringent than at level 1.

Small vs. Large Scale

6. Users will have to decide whether the small or large scale guidance is most appropriate for any given activity. There is no absolute volume cut off (such as 10 litres) to distinguish between small and large scale. The prime consideration has to be the appropriateness of the containment and control measures to the activity. It will normally be obvious which guidance (both text and tables) users should follow for their particular activity. It may even be appropriate to consider both sets of guidance for operations of an intermediate nature.

Explanation of terms

7. These explanations are not legal definitions nor are they the only possible interpretations. They are intended to aid understanding and to avoid repetition in the remainder of the text.

8. For the purpose of the guidance the terms listed below have the following meanings:

- **laboratory** - the room in which the organisms are handled, including any large scale support;
- **laboratory suite** - one or more laboratories, not necessarily of the same discipline, and ancillary rooms within a section or department with shared use of facilities such as autoclaves, centrifuges etc;
- **laboratory unit** - a separate building, or self-contained suite within a building, containing one or more laboratories and with ancillary rooms such as airlocks, changing rooms, showers, autoclave rooms etc.;
- **work place** - any area or set of areas where activities involving genetically modified organisms are undertaken, this can include laboratories, factory premises, fermentation plants, etc;
- **limit contact** - there is a general requirement to limit contact of the GMM with the general population and the environment. The extent to which contact is limited will depend on the controls in place during particular operations. The degree of control should be determined by the risk assessment and be set at a level at which the risk of harm to humans or the environment is low or negligible;
- **minimise release** - used in the context of containment and control which is to be provided (especially at large scale). It recognises that the intention is to keep the GMMs under control, but that a *limited* number of GMMs may enter the work place and/or the wider environment. In such cases the risk assessment should confirm that the risk of harm from the GMMs to humans or the environment is low or negligible. The negligible risk will usually arise because the GMMs are of low survivability in the environment i.e. they have built-in biological barriers.
- **prevent release** - used similarly to "minimise release" but it sets a higher standard of containment and control where release into the workplace and wider environment is prevented, possibly by a range of methods. It will usually be appropriate to prevent release in cases in which the GMM is highly hazardous to humans and/or the environment. The risk assessment should clearly demonstrate the efficacy of the intended control measures;
- **closed system** - a system which physically separates the process and GMMs from the environment and used in the context of large scale containment. Typically a closed system might involve the use of a fermenter vessel which prevents any significant contact of the GMMs with the open air, workplace or wider environment. The degree to which the system is "closed" should be determined by the risk assessment and for lower levels of risk the system may not need to be completely sealed. The closed system might involve "open" equipment in a safety enclosure. For any particular activity the closed system may encompass several stages and different pieces of equipment linked by a closed transfer system. For instance, a seed vessel leading to a fermentation vessel to a downstream processing stage may all be part of a single closed system. The integrity of the closed system should be routinely tested;
- **optional** - indicates that the requirement for a particular containment or control measure is to be determined based on the risk assessment. Where the risk assessment indicates that a certain measure is needed to protect human health or the environment its use is not optional.

Section I: Containment and Control Measures for Small Scale, Laboratory Activities[1]

9. In the following text, regulatory requirements are explicitly stated. The remainder of the guidance is recommended good practice and illustrates possible approaches. Alternative measures may be applied so long as they are appropriate to the level of risk. Table 1 summarises the containment and control measures for small scale activities.

10. For all containment levels there should be a reasonable amount of space provided. There is no set standard, although a figure of $24m^2$ is recommended in the ACDP publication "Categorisation of biological agents according to hazard and categories of containment. The appropriate space allocation should take account of both work practices and equipment.

[1] This may include large scale support laboratories

11. Personal protective equipment, including protective clothing, must be: stored in a well defined place; checked and cleaned at suitable intervals; and when discovered to be defective, repaired or replaced before further use. Personal protective equipment which may be contaminated by biological agents must be: removed on leaving the working area; kept apart from uncontaminated clothing; and decontaminated and cleaned or, if necessary, destroyed.

ACGM Containment Level 1

12. Laboratory personnel should receive information, instruction and training in the procedures conducted in the laboratory

Building/Physical Measures

a) There is no requirement for the laboratory to be mechanically ventilated. If it is ventilated, room air may usually be extracted to atmosphere without need for prior filtering.

b) Good hygiene should be maintained and this is aided if the laboratory is easy to clean, particularly the bench surfaces. These should be impervious to water and resistant to those acids, alkalis, solvents and disinfectants that may be expected in normal use.

c) The laboratory should contain a basin or sink that can be used for hand washing.

Work Practices

d) The laboratory door should normally be closed when work is in progress.

e) Laboratory coats or gowns should be worn in the laboratory and removed when leaving the laboratory suite.

f) Gloves should be used if indicated by the risk assessment.

g) Hands should be washed as soon as contamination is suspected, after handling viable GMMs and before leaving the laboratory.

h) Procedures should be carried out in such a way as to keep aerosol production to a minimum. Where aerosol production is unavoidable the risk assessment should determine measures to control exposure of workers and the environment.

i) Effective disinfectants should be available for immediate use in the event of spillage (see Appendix 4).

j) Bench tops and laboratory equipment should be cleaned or disinfected as appropriate after use.

k) Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner. Pipettes, if placed in disinfectant, should be totally immersed.

l) All waste material containing viable GMMs should be disposed of in a safe manner.

m) Materials for disposal should be transported without spillage in robust containers.

n) All accidents and incidents should be recorded. The Contained Use Regulations require certain accidents to be reported to the HSE. Further guidance on this can be found in the HSE publication "A guide to the Genetically Modified Organisms (Contained Use) Regulations" and in Part 1 of the Compendium.

ACGM Containment Level 2

13. Laboratory personnel should receive information, instruction and training in handling of micro-organisms, including GMMs, and an appropriate standard of supervision of the work is to be maintained. (See Part 1 of the Compendium regarding training and supervision.)

Building/Physical Measures

a) There is no requirement to separate the work place from other activities in the same building. However, there may be instances when this is advisable. (For example, it may be necessary for work with certain viruses to be carried out under relatively isolated conditions.) The risk assessment should be used to identify when partial or more rigorous separation is needed.

b) Generally there is no specific requirement to provide mechanical ventilation for containment, although this may be present as air conditioning. Where a risk assessment indicates the need for a mechanical ventilation system (for example to contain airborne pathogens where the ventilation system provides secondary containment) then the laboratory should be at a nominal negative pressure. This can be achieved by ensuring that the supply air is nominally 90% of the extract. Risk assessment will determine the requirement for HEPA filtration of the extracted air although this normally will not be necessary

c) Good hygiene should be maintained and this is aided if the laboratory is easy to clean, particularly the bench surfaces. These should be impervious to water and resistant to those acids, alkalis, solvents and disinfectants that may be expected in normal use.

d) A dedicated hand wash basin should be available for hand washing. It should be located near the laboratory exit and preferably have taps which can be operated without being touched by hand.

e) Vector control systems (for rodents and insects as appropriate) are required for animal containment. There may be instances where vector control should be implemented for laboratory containment. For instance, insect control may be needed when working with some insect borne pathogens.

Work Practices

f) Biohazard signs indicating the level of work should be displayed if the risk assessment indicates that this is necessary. (Note that it is a regulatory requirement that a biohazard sign and other relevant warning signs be displayed if the GMM is also a biological agent.)

g) Access to the laboratory should be limited to laboratory personnel and other specified persons. The nature of the work should be taken into account when considering who is to be authorised for access.

h) The laboratory door normally should be closed when work is in progress, and must be closed if the risk assessment indicates that this is necessary. (For instance where airborne pathogens are being worked on and where mechanical ventilation is in operation.)

i) Laboratory coats or gowns (preferably side or back fastening) should be worn in the laboratory. Coats should be removed before washing hands when leaving the laboratory suite. Ideally a sufficient number of separate storage pegs should be provided in the laboratory suite in order that there is no more than one coat per peg. Coats should be changed on a regular basis and immediately should they become contaminated.

j) Gloves should be worn if indicated by the risk assessment.

k) Hands should be decontaminated as soon as contamination is suspected, after handling viable GMMs and before leaving the laboratory.

l) In general (unless the risk assessment indicates otherwise) work may be conducted on the open bench but procedures which keep aerosol production to a minimum need to be used. Where aerosol production is unavoidable, for instance vigorous shaking or mixing and ultrasonic disruption etc., a suitable microbiological safety cabinet or equipment which is designed to contain the aerosol should be used, such as a centrifuge with sealed containers. Where sealed buckets or rotors are being used, the seals should be tested in accordance with BS EN 61010-2-20 (1995), as sealed containers are liable to breakage and cannot be relied upon to provide containment. Where a microbiological safety cabinet is used, care should be taken to appropriately filter extracted air, whether it is exhausted to

outside air or recirculated, where there is risk of harm from not doing so.

m) For work where the use of a Class II cabinet is proposed see Annex 3.

n) Effective disinfectants should be available for routine disinfection and immediate use in the event of spillage (see Annex 4).

o) Bench tops and laboratory equipment should be cleaned and disinfected as appropriate during and after use.

p) Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner. Pipettes, if placed in disinfectant, should be totally immersed.

q) All infected waste material should be disposed of in a safe manner. Group II GMMs are required by the regulations to be inactivated by validated means prior to disposal.

r) An autoclave for the sterilisation of waste materials should be readily accessible. The autoclave would usually be in the same building as the laboratory. Alternative methods, including removal by contractors for remote disposal (e.g. incineration) may be acceptable. It should be noted that the contractors and disposal site/incinerator will need to comply with the Contained Use Regulations if viable GMMs are handled, e.g. notification of premises.

s) Materials for disposal should be transported without spillage in robust leakproof containers. If waste is to be taken off site the Carriage of Dangerous Goods (Classification and Packaging and Labelling) Regulations will apply - see Part I of the Compendium for further information.

t) All accidents and incidents should be recorded and immediately reported to a competent person. The Contained Use Regulations require accidents to be reported to the HSE (see Part 1 for further guidance).

ACGM Containment Level 3

14. Laboratory personnel should have had information, instruction and training in handling of micro-organisms. A high standard of supervision of the work needs to be maintained. (See Part 1, concerning training and supervision.)

Building/Physical Measures

a) The laboratory should be separated from other activities in the same building, and should be in an area away from general circulation. The degree of isolation should be appropriate to the risk.

b) A continuous airflow into the laboratory should be maintained when work is in progress. As a guide, 8-10 air changes/hour are often used, however the rate will vary depending on the nature of the facility. The only requirement is that the workplace should be maintained at an air pressure negative to the surrounding atmosphere. "Atmosphere" in this context can be interpreted as outside the building or in adjacent parts of the laboratory suite or building. It is normal to reference pressure difference to a point within the building to avoid wind effects, and to ensure that the system accommodates building leakage.

c) Possible methods of achieving appropriate air flow include once through ventilation systems with HEPA filtration before the point of extract. Where microbiological safety cabinets are used then they should be HEPA filtered before discharge. A combination of microbiological safety cabinets and ventilation system is common.

d) It is good practice to make provisions for comfort factors e.g. fresh air, reasonable temperature and humidity control.

e) In laboratories which have a mechanical ventilation supply, the supply and extract airflow should be interlocked to prevent positive pressurisation of the room in the event of failure of the extract fan. If the supply fails, the extract air can continue, but if the extract fails, the supply should be tripped off. Where there is a combination of microbiological safety cabinets and ventilation within an area, then the design of the ventilation system should prevent the pressurisation of the laboratory when the cabinets fail or are switched off.

f) A high standard of hygiene should be maintained and this is more easily achievable if the laboratory is easy to clean. In particular the bench surfaces and floor should be impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants that may be expected in normal use.

g) A dedicated hand wash basin should be available for hand washing and located near the laboratory exit. Taps should be operated without being touched by hand.

h) There should be an observation window, or alternative, so that occupants can be seen. A glass panel in the door is often sufficient.

i) Vector control systems (for rodents and insects as appropriate) are required for containment of animals infected with GMMs which are also biological agents. There may be instances where vector control is also needed for laboratory containment. For instance, insect control when working with insect borne pathogens.

j) The laboratory should be sealable to permit fumigation particularly when a major spillage has occurred or prior to maintenance. (It is to be noted that this is a requirement for biological agents in Hazard Group 3. See Annex IV).

k) Although not normally required, where the risk assessment indicates that it is necessary effluent from sinks and showers (if any) needs to be collected and inactivated before release.

Work Practices

l) Biohazard signs indicating the level of work undertaken should be displayed at the entrance to the laboratory or laboratory suite.

m) Access to the laboratory should be limited to authorised personnel. This is often aided by the use of key coded entry systems or a signing in and out procedure.

n) The laboratory door is to be closed when work is in progress and locked when the room is unoccupied. Key holders should be trained workers only.

o) Laboratory coats or gowns (side- or back-fastening) should be worn in the laboratory. Coats should be removed before washing hands when leaving the laboratory suite and should not be used outside the laboratory suite. A sufficient number of

separate storage pegs should be provided in the laboratory suite in order that there is no more than one coat per peg. Coats should be changed on a regular basis and immediately should they become contaminated and should be autoclaved before removal for laundering.

p) Gloves should be worn for all work with infective materials. Gloves should be washed or preferably removed before touching items that will be touched by others not similarly protected, e.g. telephone handsets, paperwork.

q) The laboratory should contain its own equipment, such as: centrifuge in which sealed buckets or sealed rotors are used, incubator, refrigerator, deep freeze, vapour phase liquid nitrogen chest etc., so that all viable materials requiring level 3 containment are stored and worked on within the laboratory and nowhere else. However there may be instances (such as an inoculum facility for large scale production) where this is not reasonably practicable. In such cases material should be transported and stored without spillage in properly labelled robust containers which are opened only in containment level 3 accommodation.

r) It is good practice for all laboratory procedures with viable material to be conducted in a microbiological safety cabinet (Class I or Class II BS5726:1992, or unit with equivalent protection factor or performance) and this is a regulatory requirement where aerosols may be produced. For work where the use of a Class II cabinet is proposed see Annex II.

s) For tissue culture work with organisms with an airborne route of transmission, or where a high degree of uncertainty exists (for example, with viruses with altered tissue tropism), it may be necessary to use a Class III cabinet. Exceptions to this are where the equipment to be used provides containment of the potential aerosol.

t) Non-re-circulating microbiological safety cabinets need to exhaust through a HEPA (or equivalent) filter to the outside air or to the laboratory air extract system. Siting, performance protection factor and air filtration should comply with the specifications detailed in BS 5726: 1992. Where re-circulating Class II cabinets are used the recirculated exhaust air should be passed through two HEPA filters in series. In these cases the maintenance of a continuous airflow into the laboratory during work will be of particular importance. Where recirculating cabinets are used consideration should be given to heat and humidity build up and fumigation procedures. If the use of recirculating cabinets are proposed, HSE should be consulted prior to the installation of the cabinets.

u) Hands should be washed as soon as contamination is suspected, after handling viable GMMs and before leaving the laboratory even if gloves are worn.

v) Effective disinfectants should be available for routine disinfection and immediate use in the event of spillage (see Annex III).

w) Bench tops and laboratory equipment should be cleaned and disinfected as appropriate after use.

x) Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner. Pipettes, if placed in disinfectant, should be totally immersed.

y) All infected waste material should be disposed of in a safe manner. Group II GMMs must be inactivated by validated means prior to disposal.

z) It would normally be expected that an autoclave for the sterilisation of waste materials be in the level 3 laboratory. If this is not possible the next best option is that there is one in the laboratory suite. Although it is permissible for waste materials to be inactivated by chemical means prior to disposal, it is normally more appropriate to autoclave waste. Where chemical disinfection is used, the disinfection procedures must be validated under working conditions, such as in the presence of buffering solutions or proteins. Details of the proposed means of waste management must be supplied to HSE as part of a Group II notification. Where incinerators are available, waste may be incinerated following safe transport.

aa) Materials for inactivation should be transported without spillage in robust leakproof containers.

ab) Where the risk assessment indicates that it is necessary, personnel should shower before leaving the laboratory.

ac) All accidents, spills and exposures to infective materials should be immediately

reported to and recorded by a competent person. The regulations also require that all accidents are reported to HSE (see Part 1 of compendium for guidance).

ACGM Containment Level 4

15.The guidance in this section assumes a requirement to protect both the operator and the environment. It is recognised, however, that work with certain animal or plant pathogens may require a very high level of environmental protection, and less operator protection. In these types of facilities, the whole laboratory block is considered to be at level 4 for environmental protection, although the worker may operate on the open bench, or in an open fronted cabinet. Such containment is generally described as MAFF Containment Level 4, and is reserved for work with such organisms as Foot and Mouth Disease Virus, and Rinderpest virus. Anyone proposing to work with such viruses should contact MAFF (see Part 2B, Annex II).

Specific rules should be drawn up for the work in the laboratory. Personnel should have specific training in the working of the laboratory and use of safety equipment and information on the handling of the micro-organisms concerned. The work should be supervised.

Building / Physical Measures

a) The laboratory unit should be a separate building or form an isolated part of a building.

b) The laboratory should be ventilated by "once through" systems giving a minimum air change rate of 20 air changes per hour. The extract system should have 2 stages of HEPA filtration in series before discharge. The supply should be fitted with one stage of HEPA filtration at the inlet. HEPA filters on inlets should be protected with panel and bag filters. Maintenance of a negative pressure of about 7mm of water in the laboratory and about 3mm of water in the air lock (or changing room/lobby) is acceptable. An alarm is to be displayed which can be read from both inside and outside the laboratory.

c) The supply and extract airflow should be interlocked to prevent positive pressurisation of the laboratory in the event of failure of the extract fan and an emergency source of electric supply should be provided to cut in automatically in the event of a power failure. The ventilation system should incorporate a means of preventing reverse airflows.

d) A scrupulously high standard of hygiene should be maintained. The laboratory unit should be designed so that it is easily cleaned. Bench surfaces, floors, walls and ceiling should be impervious to water and resistant to acids, alkalis, solvents and disinfectants.

e) An observation window, or suitable alternative, should be present in the laboratory so that the occupants can be seen.

f) Effective vector control systems (for rodents and insects) are required for animal and laboratory containment.

g) The laboratory must be sealable to permit fumigation.

h) Effluent from sinks and showers should be collected and inactivated so that it is safe for discharge

i) There should be a telephone or other means or outside communication inside the laboratory unit. Preferably with 'hands-off' controls. A fax or computer link can be useful for safe transfer of data.

Work Practices

j) Biohazard signs specifying the level of work undertaken are to be displayed on the outer door to the laboratory unit, together with a 'work in progress' sign.

k) Access to the laboratory must be restricted to authorised personnel and a key procedure established so that entry is restricted at all times. There is to be a second competent person in the laboratory unit available to assist in case of an emergency at all times when the unit is occupied.

l) Entry must be through an airlock. The clean side of the airlock is to be separated from the restricted side by changing and showering facilities and preferably by interlocking doors.

m) A complete change of clothing is to be worn in the laboratory unit. The clothing is to be removed after work in the dirty side of the changing area and placed in a container for autoclaving. High performance respiratory protective equipment (two or more units) need to be available in the clean side of the laboratory unit for use in an emergency.

n) Procedures for emergency evacuation (for example in case of a fire alarm or for removing a stretcher case) should be drawn up.

o) The laboratory must contain its own equipment. An additional ventilated airlock that can be fumigated may be required for passage of equipment which cannot enter the laboratory unit through the autoclave or personnel airlock. No equipment may be removed without disinfection by validated means.

p) All laboratory procedures with viable materials must be conducted in a class III microbiological safety cabinet (BS 5726: 1992, or one with equivalent performance). The exhaust from the cabinet should pass through two HEPA filters mounted in series before ducting to the outside air or to the laboratory air extract system. A recirculation system may also be appropriate.

q) All viable material requiring level 4 containment must be stored in the laboratory unit and nowhere else.

r) Effective disinfectants should be available for immediate use (see Annex III).

s) All material must be made safe before being removed from the laboratory unit including samples. A double-ended dunk tank filled with an effective disinfectant, or an alternative safe system , may be required for the removal of materials which cannot be autoclaved. Removal of material in this manner, and also of materials removed through the equipment airlock, should be undertaken only with authorisation of the responsible/competent person and under conditions defined in the local code of practice. The dunk tank should be sealed during fumigation if the disinfectant is incompatible with the fumigant.

t) A double-ended autoclave with interlocking doors with entry in the laboratory and exit in a clean area should be provided.

u) Personnel must shower, including hair, before leaving the laboratory unit.

v) All accidents, spills and accidental exposures to viable materials are to be immediately reported to and recorded by the competent person/person responsible who needs to take appropriate measures specified in the local rules. The Contained Use Regulations require any accident to be notified to HSE. (See Part 1 for guidance.)

Section II: Containment and Control Measures for Large Scale Activities

16. The containment and control measures for large scale activities are summarised in Table 2. In the following text regulatory requirements are clearly stated. The remainder of the guidance is either recommended good practice or examples of the sorts of methods that can be used. This guidance does not preclude the use of other approaches. Where there is no specific requirement alternative methods may be applied so long as the risks are adequately controlled.

ACGM Containment Level B1

17. The legislation does not specify particular containment and control measures for level B1. Measures described for this level are therefore indications of good practice not regulatory requirements.

18. That said, there is a requirement for the principles of good microbiological practice and good occupational safety and hygiene to be applied (see also Part 3 , para 22 to 24) at all containment levels. It is also a requirement that containment be used to limit the GMM's contact with the general population and the environment, even though many GMMs appropriate to be handled at level B1 present little risk of harm to human health or the environment.

19. It is recognised that for the lower risk GMMs the containment for process requirements (or good manufacturing practice) is often more stringent than that needed for human health or environmental safety. The degree to which their contact with humans and the environment needs to be limited will vary and should be based on the risk assessment.

Building Design

a) There is no need for activities to be undertaken within controlled areas, however, it is normally sensible for production or factory floor areas to be separated from offices, laboratories and other facilities.

b) Good hygiene is advisable and buildings designed or adapted for easy cleanability will aid this.

c) Mechanical ventilation is not normally needed although it may be used for the removal of heat from process operations and for worker comfort. For some processes a positive air pressure is needed to maintain product integrity. This is acceptable so long as it does not conflict with any need to control organisms within the facility in the event of an accident. The use of localised airflow units could be considered which give product and operator protection.

d) It is not normally necessary to design the facility to contain spillage of the contents of the closed system.

Fermentation Methods, Equipment and Utilities

e) Viable GMMs should be contained in a system which includes physical barriers to separate them from the general environment (but see comments about waste disposal below). This is not necessarily a "closed" system, for example brewing processes may utilise GMMs in open vessels, however, the vessel provides a general containment of the contents. The need for a closed system will depend upon the risk assessment but for most activities at B1 such a system would not normally be needed. For many activities at level B1 suitable physical barriers could be provided by the building itself. Most of the normal equipment used in the particular industry would be suitable for work at level B1 - such as ventilated flasks, open top fermenters , open mixing vessels, baking tins and trays, moulds etc.

f) Release of GMMs into the work place and wider environment should be minimised during procedures such as the addition of materials, mixing or transfer of GMMs between vessels so as to limit the GMMs contact with people and the environment. The acceptable degree of minimisation is to be determined by the risk assessment. Where there is no risk of harm elaborate methods for controlling escape are unlikely to be necessary.

g) Where seals are used on equipment they should be designed to minimise release so that contamination of the workplace and

wider environment is limited appropriately and harm will not result.

h) It is not always necessary to inactivate bulk culture fluids containing Group I (i.e. not Group II) GMMs before they are removed from containment. However, if the risk assessment indicates that harm may result if viable GMMs are released they should be inactivated. (See waste handling below.

Maintenance

i) Equipment and control measures should be tested and maintained at appropriate intervals.

Management Systems / Work Practices

j) Workers should be appropriately trained in both routine and emergency procedures. Written operating instructions, including where necessary emergency plans and spillage policy, are recommended. (See Part 1, concerning training and supervision.)

k) Washing facilities should be provided for personnel.

l) Work clothing should be provided as necessary.

Sampling Procedures

m) Release of GMMs into the work place should be minimised during sample collection.

Waste Handling and Gas Emission

n) Waste should be disposed of in a safe manner.

o) There is no need to treat exhaust gases

Accidents/Emergency Plans

p) There is normally no need for emergency plans, although it is good practice to have procedures drawn up to deal with spillages.

q) Accidents and incidents are to be recorded. All accidents are required to be notified to HSE (see Part 1 for further guidance).

Monitoring

r) Monitoring is unlikely to be required for many activities at level B1. However, where there is a risk to human health or environmental safety from process organisms outside the closed system, monitoring for viable process organisms should be carried out.

ACGM Containment Level B2

20.See Table 2 for a summary of containment and control measures at Level B2.

Building Design

a) Where indicated by the risk assessment activities should be undertaken within controlled areas which are separated from offices, laboratories and other facilities, and where cross traffic is limited.

b) Good standards of hygiene should be maintained and this can be aided by good building design. Buildings will usually be of normal industrial construction with sealed impervious floors and standard industrial wall cladding. Floors can be sprayed with epoxy resin or constructed of non-porous concrete to aid cleanability. Walls and ceilings can also be sprayed with epoxy resin or covered with resin bonded fibre. If welded vinyl is used care needs to be taken as joints can open in negative air pressure conditions.

c) Where indicated by the risk assessment the controlled area should be ventilated to minimise air contamination. Mechanical ventilation may also be used for worker comfort (about 10 changes per hour is normal). Although it is not normally necessary to maintain an air pressure negative to atmosphere, where mechanical ventilation is used this would be considered to be good practice. Typical pressure differentials would be 1 - 5 mm water, although it is considered to be more important to ensure appropriate flow of air than to maintain any particular pressure differential.

d) HEPA (or equivalent) filtration of any input and extract air is not usually needed, but filtration of extract air may be necessary particularly where there is a risk to the wider environment from the GMM.

e) Where there is risk of harm from catastrophic (total) loss of containment the facility should be designed to contain spillage of the entire contents of the fermenter. Some possible approaches include bunding, enlarged drainage channels and/or drainage to a kill tank. The containment method employed should also allow for inactivation to be undertaken.

Fermentation Methods, Equipment and Utilities

f) Viable GMMs should be contained in a closed system which includes physical barriers to separate them from the work and wider environment. The design of equipment should be appropriate to the risk assessment. Fermenters will usually be stainless steel and flanged with welded pipework, but other arrangements may also be suitable.

g) Pipework and valves should be designed with the emphasis on leak-tightness as well as on cleanability. Connection of services to equipment should consider prevention of back flow. This can be alleviated by a pressure differential. If back contamination is a problem steam locks and "double block and bleed" systems may be considered. Non-return valves may be unreliable from a microbiological point of view and their use should be considered very carefully. Addition of materials to the closed system and transfer of viable GMMs to another closed system should be performed so as to minimise release. It is preferable for all potentially contaminated liquids to be transferred in closed piping. Where media/culture pipework and equipment are glass or plastic rather than steel care should be taken to avoid them being subject to positive pressure.

h) Inoculation of seed vessels can be by direct injection using a sterile needle/septum technique or by using a closed system with a steel transfer vessel or similar. Where the needle/septum method is used procedures should be carefully thought out to avoid needle puncture injury.

i) Static seals on equipment should be designed so as to minimise release. Examples of typical types of seals appropriate for most level 2 work are:

- single "O" ring seals;
- flat gasket;
- dairy-type sealed couplings;
- sanitary couplings with gaskets.

j) Agitator seals would normally be single or double faced mechanical seals. Where necessary seals can be enclosed in HEPA (or equivalent) filtered housings. These examples are not exhaustive. Other alternatives may be used if they are appropriate.

k) Fixed or retractable instrument sensors may be used.

l) Any pressure relief systems design needs to be considered carefully. Possible methods can include chains of venting vessels. It will be necessary to assess the operating pressure and the risk of the GMM. Pressure Vessels Regulations requirements must be met.

m) Bulk culture fluids should not be removed from the closed system unless the viable GMMs have been inactivated by validated means. Chemical or physical methods are acceptable.

Maintenance

n) Equipment and control measures should be tested and monitored. Fermenters are normally leak tested. This can be on commissioning and before every run and after a major engineering change or maintenance. Leak testing can employ for example, the use of compressed air, water, vacuum or tracer gases such as SF_6. Good design can aid maintenance. Grey side technology, with separation of the maintenance side from the process side, can be beneficial. It may be necessary for some maintenance to be performed by trained production staff to limit exposure of maintenance personnel.

Management Systems/Work Practices

o) Workers should be appropriately trained in both routine and emergency procedures. There should be written operating instructions, including where necessary emergency plans and spillage policy.

p) Access should be restricted to nominated personnel when this is indicated by the risk assessment. Control can be effected by a pass system, dress code, card keys, digital locks or similar alternative methods.

q) Hand washing facilities, ideally with foot or elbow operated taps, should be provided for personnel together with disinfectant soap. Emergency showers and eye wash stations are useful.

r) Where indicated by the risk assessment, biohazard signs should be posted at entrances. (This is a requirement for all work with biological agents.)

s) Work clothing should be provided and ideally is to be kept in a separate locker. Where indicated by the risk assessment the clothing should be decontaminated before laundering.

Sampling Procedures

t) Release of GMMs into the work place should be minimised during sample collection. Where indicated by the risk assessment samples should be taken using aseptic techniques. These will often involve sterilising the sampling connection. The receiving container should be designed to minimise aerosols. Running directly to drain for a mid-stream sample is not acceptable. This material must be collected and decontaminated.

Waste Handling and Gas Emission

u) Infected waste and effluent containing viable GMMs should be inactivated by validated means prior to final discharge. This might be achieved *in situ* or by means of separate kill tanks. Chemical or physical methods of inactivation may be used, but, for chemical treatment especially, the constituents of the waste should be considered when deciding on the appropriate method. Waste discharge will need to comply with all relevant legislation under the Environmental Protection Act 1990. It is advisable to consult the relevant authorities at an early stage.

v) Exhaust gases should be treated so as to minimise release. Various methods are available for treatment of off gases, these might include: filtration (0.2 µm maximum for total filtration); the use of a cyclone separator either by itself or followed by a spray tower where gases are in contact with hypochlorite spray; impingement filters; off-gassing through chemical disinfectants. This list of examples is not exhaustive and other techniques may be used as appropriate. More than one method may be used in combination. For example a cyclone separator or impingement filters may be used as a pre-stage to help maintain the integrity of the main filters. It is important to keep filters dry to maintain their efficiency.

w) Filters should be able to be removed safely for protection of maintenance engineers.

Accidents/Emergency Plans

x) If the risk assessment indicates that, as a result of any foreseeable accident, the health and safety of persons outside the premises may be affected or if there is any risk to the environment, an emergency plan must be drawn up. Detailed guidance on what this should contain can be found in guidance to the Contained Use Regulations[2]. Whether or not a formal emergency plan is required it is good practice to have procedures drawn up to deal with spillages.

y) Accidents and incidents should be recorded and immediately reported to a competent person. Accidents are also required to be notified to HSE (see Part 1).

Monitoring

z) Where there is risk to human health or environmental safety from process organisms outside the closed system monitoring for viable process organisms should be carried out.

[2] 'A guide to the Genetically Modified Organisms (Contained Use) Regulations'. ISBN0-7176-1186-8.

ACGM Containment Level B3

21. See Table 2 for a summary of containment and control measures for Level B3.

Building Design

a) Most activities undertaken at level B3 should be undertaken within controlled areas which are separated from offices, laboratories and other facilities and which are away from general circulation routes.

b) High standards of hygiene should be maintained and this will be aided by good building design. It should be noted that industrial wall cladding is not recommended.

c) Where the risk assessment indicates it a continuous airflow into the facility should be maintained when work is in progress. A minimum of 8-10 air changes per hour is recommended, and the workplace should be maintained at an air pressure negative to atmosphere. "Atmosphere" in this context can be interpreted as outside the building or in adjacent parts of the laboratory suite or building. It is normal to reference pressure difference to a point within the building to avoid wind effects, and to ensure that the system accommodates building leakage.

   It is good practice to make provisions for comfort factors e.g. fresh air, reasonable temperature and humidity control.

d) Extract air is normally filtered through HEPA filter and must be filtered when there is a risk of harm from not doing so. Care should be taken that contaminated air can not be drawn into the ventilation inlets of another installation. The appropriateness of recirculating air will depend on validated filter efficiency, but is generally not recommended.

e) Inlet and extract systems can be alarmed, interlocked and indicated. It is also worth considering the use of dynamically controlled variable speed fan motors to compensate for filter blocking.

f) Where indicated by the risk assessment the controlled area should be sealable to permit fumigation.

g) The facility should be designed to contain spillage of the entire contents of a fermenter. The method employed should allow for chemical or physical inactivation of the GMMs. The use of open drainage channels is not recommended at level B3.

Fermentation Methods, Equipment and Utilities

h) Viable GMMs should be contained in a closed system which includes physical barriers to separate them from the general environment. The design of the equipment should be appropriate to the risk assessment and will be similar to that used at level B2. However, addition of materials to the closed system and transfer of viable micro-organisms should be performed so as to prevent release. Seals should be designed to prevent release. Although suitable alternatives may be used it is recommended that:

   - all pipework be welded wherever practical;

   - agitator seals are double faced mechanical seals with condensate fed to the interspaces (ideally with the condensate temperature being monitored and alarmed);

   - especial care is taken to avoid contaminating utilities;

   - retractable sensors are not used - duplicate sensors being safer where there is high risk.

i) Inoculation of seed vessels should be performed so as to prevent release and closed systems such as stainless steel transfer vessels should be used. Use of sterile needle/ septum techniques is not recommended for level B3.

j) Bulk culture fluids should not be removed from the closed system unless the viable GMMs have been inactivated by validated chemical or physical methods.

Maintenance

k) See guidance to level B2. Leak testing at level B3 is normally performed using halogens.

Management Systems/Work Practices

l) Workers need to be trained to a high standard in both routine and emergency procedures. There should be written operating instructions, including any emergency plans and spillage policy. (See Part 1 concerning training and supervision.)

m) Access should be restricted to nominated personnel. The access route can be via changing rooms and a system of control which prevents unauthorised access should be in place. This may involve such things as card keys or digital locks or similar.

n) Hand washing facilities should be provided, preferably with foot or elbow operated taps. Emergency showers and eye wash stations are worth considering. Where the risk assessment indicates that it is necessary personnel should shower before leaving the controlled area. If indicated by the risk assessment effluent from the sinks and showers should be collected and inactivated before discharge.

o) Biohazard signs should be posted at access points.

p) Protective clothing should be worn and a change should be provided on each entry into the controlled area. Clothing should be decontaminated before laundering. Care should be taken to bag or otherwise contain the clothing for transport to the autoclave.

q) Consideration could be given to transferring data from the containment area by electronic means.

Sampling Procedures

r) Sampling should be performed using a closed aseptic technique and release of GMMs should be prevented.

Waste Handling and Gas Emission

s) See guidance for level B2, but note that for level B3:

- exhaust gases should be treated so as to prevent release. This will normally involve HEPA (or equivalent) filtration with 0.2 µm filter cartridges. It is often useful to have two filters in series. Care should be taken to avoid HEPA filters getting wet as this reduces their efficacy. Filters should be able to be removed safely;

- spray towers, cyclone separators, off-gasing through chemical disinfectants and impingement filters are not recommended at level B3.

Accidents / Emergency Plans

t) It would normally be expected that an emergency plan will be drawn up for level B3 and one must be made where the risk assessment indicates a reasonably foreseeable accident that may harm the health and safety of persons outside the premises or the environment. Detailed guidance on what the plan should contain can be found in guidance to the Contained Use Regulations. The emergency plan should include procedures to deal with spillages.

u) Accidents, spills and exposures to infective material need to be immediately reported to and recorded by a competent person. Accidents are also required to be reported to HSE (see Part 1).

Monitoring

v) There would normally be a risk to human health or the environment from process organisms from level B3. Where this is the case, monitoring for viable process organisms should be carried out.

ACGM Containment Level B4

22. Large scale level B4 facilities are very rare and extremely specialised. The guidance here is meant to be only an outline of the regulatory requirements and users are advised to seek advice from HSE at an early stage should they wish to construct such a facility.

Building Design

a) Activities at level B4 must be undertaken within purpose built controlled areas which are physically separated from any other activity.

b) Scrupulous levels of hygiene are to be maintained and this needs to be taken into account in the design.

c) The controlled area must be ventilated to minimise air contamination and an air pressure negative to atmosphere must be maintained. A typical pressure differential might be 1.5 mm water between the changing room/entry lobby and the work area and 1.5 mm water between the entry lobby and outside the facility. The pressures should ensure that air goes through filters effectively.

d) Input and extract air should be filtered through HEPA filters. A single filter should be used on input air to prevent adventitious contamination if there is failure of ventilation. Extract air should be filtered through two HEPA filters mounted in series.

e) The input and extract airflow should be interlocked to prevent positive pressurisation in the event of failure of the extract fan. The ventilation system should be alarmed, with a system to prevent reverse airflows and an emergency electric supply.

f) The controlled area must be sealable to permit fumigation.

g) The controlled area must be designed to contain the entire contents of the fermenter and allow for physical inactivation. Drainage channels are not appropriate at level B4.

Fermentation Methods, Equipment and Utilities

h) Viable GMMs must be contained in a fully closed system which prevents release. Especial care should be taken in the design of seals and pipework.

i) Any addition of materials to the closed system or transfer of viable GMMs to another closed system must be performed so as to prevent release. The use of sterile needle / septum techniques should not be used for inoculation at Level B4.

j) Before bulk culture fluids are removed from the closed system the viable GMMs must have been inactivated by validated means.

Maintenance

k) Equipment and control measures must be tested and maintained at appropriate intervals.

Management Systems/Work Practices

l) Workers must have specific training in working in the facility including the use of safety equipment and handling of the GMMs concerned. Specific rules should be drawn up.

m) Access must be restricted to authorised persons only. Entry must be via a changing room/lobby area (airlock) which is itself ventilated and maintained at an air pressure negative to outside the facility, but positive with respect to the work area. The entrance needs to be kept locked.

n) Decontamination and washing facilities must be provided. Personnel must shower before leaving the controlled area. Effluent from the sinks and showers must be collected and inactivated before discharge.

o) Biohazard signs must be posted at the entrance.

p) A complete change of protective clothing must be worn a change being provided for each entry. Clothing must be decontaminated before being removed from the controlled area for laundering.

q) Consideration should be given to transferring data from the containment area by electronic means.

Sampling Procedures

r) Only closed aseptic techniques are acceptable when taking samples. Release of GMMs must be prevented.

Waste Handling and Gas Emission

s) All effluent must be inactivated by validated physical means prior to final discharge.

(Chemical methods should not be used as they are less easy to validate and more subject to variation.)

t) All exhaust gases must be HEPA filtered to prevent release.

Accidents / Emergency Plans

u) Emergency plans must be drawn up and include procedures to deal with spills.

v) Accidents, spills and exposures to infective materials are to be immediately reported to and recorded by the competentperson/person responsible who needs to take appropriate measures specified in the local rules. Accidents are required to be reported to HSE (see also Part 1).

Monitoring

w) A monitoring programme should be instigated in the work area and immediate surroundings.

TABLE 1

Containment and Control Measures for Small Scale Activities

This table summarises systems which have been found to be suitable. It should be read in conjunction with paragraphs 12 to 15 of Part 3A which expands on items in the Table and also Part 3 which discusses more general requirements.

The measures for a particular activity should be chosen according to the risk assessment. It is acceptable to use measures from more than one containment level for an activity if this is in accordance with the risk assessment.

Biological Agents

The measures set out are equivalent to those required for work with biological agents under COSHH 1994. It should be noted that COSHH sets minimal standards for handling biological agents in Hazard Groups 2, 3 and 4. Further guidance can be found in Part 1. You are also advised to consult the ACDPs "Categorisation of biological agents according to hazard and categories of containment" for further detail about the requirements for working with biological agents. **Table cells have been shaded to indicate where measures also feature in COSHH.**

Containment and Control Measures	Containment Level 1	Containment Level 2	Containment Level 3	Containment Level 4
Building / Physical Measures:				
The workplace separated from other activities in the same building	No	No	Yes	Yes
The workplace maintained at an air pressure negative to atmosphere[1]	No	No, unless mechanically ventilated	Yes	Yes
Input air and extract air to the workplace are to be filtered using HEPA or equivalent	No	No	Yes, on extract air	Yes, on input and double (2 stage in series) on extract air
Surfaces impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants	Yes, for bench	Yes, for bench	Yes, for bench and floor (and walls for animal containment)	Yes, for bench, floor, walls and ceiling
An observation window, or alternative present so that occupants can be seen	No	No	Yes	Yes
Efficient vector control eg rodents and insects	No	Yes, for animal containment	Yes, for animal containment	Yes
The workplace sealable to permit fumigation	No	No	Yes	Yes

Containment and Control Measures	Containment Level 1	Containment Level 2	Containment Level 3	Containment Level 4
Effluent from sinks and showers collected and inactivated before release	No	No	Optional	Yes
Work Practice Measures:				
Biohazard signs and level of work posted	No	Optional[2]	Yes	Yes
Access restricted to authorised persons only	No	Yes	Yes	Yes
Personnel trained in both routine and emergency procedures	Yes	Yes	Yes	Yes
Laboratory door closed when work is in progress	Optional	Optional	Yes, should be locked when room is unoccupied	Yes, door to be kept locked
Personal protective equipment protective clothing gloves RPE	 Yes Optional No	 Yes Optional No	 Yes Optional No	 Yes Yes Yes
Protective clothing decontaminated before laundering	No	Optional	Yes	Yes
Smoking, eating, drinking and the application of cosmectics prohibited in workplace	Yes	Yes	Yes	Yes
Laboratory to contain its own equipment	No	No	Yes, so far as is reasonably practicable	Yes
Equipment and control measures tested and maintained	Yes	Yes	Yes	Yes
Viable material, including any infected animal, to be handled in biological safety cabinet or isolator or other suitable container	No	Yes, where aerosol produced	Yes, where aerosol produced	Yes (Class III cabinet)
Monitoring for the relevant organisms outside	Optional	Optional	Yes	Yes
Safe storage of GMMs	Yes	Yes	Yes	Yes, secure storage

Containment and Control Measures	Containment Level 1	Containment Level 2	Containment Level 3	Containment Level 4
Contaminated waste to be inactivated prior to disposal	Optional	Yes, by validated means	Yes, by validated chemical or physical means	Yes, by validated physical means
Autoclave available in the laboratory	Optional	Optional	Optional	Yes, double ended
Incinerator for disposal of animal carcasses	Optional (for animal containment)	Accessible (for animal containment)	Accessible (for animal containment)	Yes, on site (for animal containment)
Decontamination and washing facilities provided	Yes	Yes	Yes	Yes
Personnel shower before leaving the laboratory	No	No	Optional	Yes

[1] "Atmosphere" is not necessarily the atmosphere outside the laboratory. See the main text for further discussion

[2] Optional indicates that the requirement is to be determined based on the risk assessment

TABLE 2

Containment and Control Measures for Large Scale Activities

This table summarises systems which have been found to be suitable. It should be read in conjunction with paragraphs 16 to 22 of Part 3A which expands on items in the Table and also Part 3 which discusses more general requirements.

The measures for a particular activity should be chosen according to the risk assessment. It is acceptable to use measures from more than one containment level for an activity if this is in accordance with the risk assessment.

Note that the provisions of Table 1 apply to the preparation of seed cultures and for process control laboratories associated with large scale activities.

Biological Agents

The measures set out are equivalent to those required for work with biological agents under COSHH 1994. It should be noted that COSHH sets minimal standards for handling biological agents in Hazard Groups 2, 3 and 4. Further guidance can be found in Part 1. You are also advised to consult the ACDPs "Categorisation of biological agents according to hazard and categories of containment" for further detail about the requirements for working with biological agents. **Table cells have been shaded to indicate where measures also feature in COSHH.**

Containment and Control Measures	Containment Level B1	Containment Level B2	Containment Level B3	Containment Level B4
		Building Design:		
Closed systems located within a controlled area	(Not applicable)	Optional	Optional	Yes, and purpose built
The controlled area adequately ventilated to minimise air contamination	No	Optional	Optional	Yes
The controlled area maintained at an air pressure negative to atmosphere	No	No	Optional	Yes
Input and extract air to the controlled area HEPA filtered	No	No	Optional	Yes
The controlled area sealable to permit fumigation	No	No	Optional	Yes
The controlled area designed to contain spillage of the entire contents of the closed system	No	Optional	Yes	Yes

Containment and Control Measures	Containment Level B1	Containment Level B2	Containment Level B3	Containment Level B4
Fermentation Methods, Equipment and Utilities:				
Viable micro-organisms contained in a system which physically separates the process from the environment (closed system)	No	Yes	Yes	Yes
Addition of materials to a closed system and transfer of viable micro-organisms to another closed system performed so as to:	(Not applicable)	Minimise release	Prevent release	Prevent release
Equipment seals designed so as to:	Minimise release (if seals used)	Minimise release	Prevent release	Prevent release
Bulk culture fluids not removed from the closed system unless the viable micro-organisms have been	(Not applicable)	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated chemical or physical means
Maintenance				
Equipment and control measures tested and maintained	Yes	Yes	Yes	Yes
Management Systems/Work Practices:				
Personnel to be trained in both routine and emergency procedures	Yes	Yes	Yes	Yes
Access restricted to nominated personnel only	No	Optional	Yes	Yes, via airlock
Decontamination and washing facilities provided for personnel	Optional	Yes	Yes	Yes
Personnel shower before leaving the controlled area	No	No	Optional	Yes
Biohazard signs posted	No	Optional	Yes	Yes
Personnel wear protective clothing	Yes, work clothing	Yes, work clothing	Yes	Yes, a complete change
Protective clothing decontaminated before laundering	No	Optional	Yes	Yes
Smoking, eating, drinking and the application of cosmetics prohibited in controlled areas	Yes	Yes	Yes	Yes

Containment and Control Measures	Containment Level B1	Containment Level B2	Containment Level B3	Containment Level B4
Sampling Procedures:				
Sample collection performed so as to:	Minimise release	Minimise release	Prevent release	Prevent release
Sampling by closed aseptic technique	No	Optional	Yes	Yes
Waste Handling and Gas Emission:				
Effluent from sinks and showers collected and inactivated before release	No	No	Optional	Yes
Effluent treatment before final discharge	Optional	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated physical means
Exhaust gases from the closed system treated (so as to)	(Not applicable)	Minimise release	Prevent release	Prevent release
Accidents / Emergency Plans:				
Emergency plans prepared	No	Optional	Yes	Yes
Documented spillage procedures drawn up	Optional	Yes	Yes	Yes
Monitoring:				
Monitoring for process organisms outside primary containment	Optional	Optional	Yes	Yes

[1] Optional indicates that the requirement is to be determined based on the risk assessment

Annex I

CONTAINMENT AND CONTROL MEASURES FOR WORK WITH NAKED ONCOGENIC DNA

1. The following guidance sets out the specific control requirements under COSHH that may apply to work with oncogenic sequences handled as preparations of naked DNA. The definition of a carcinogen for the purposes of COSHH is explained in Part 1B and further guidance is in Part 2B. ACGM endorses the recommendation in the COSHH General ACoP to adopt a precautionary approach where there is uncertainty about the status oncogenic DNA sequence as a carcinogen. In any case, irrespective of whether naked oncogenic DNA is a carcinogen as defined, it is arguably a substance hazardous to health and the general provisions of COSHH will apply.

2. Oncogenes present in GMOs should be assessed and controlled as set out in Part 2A (for bacteria and cell cultures) 2B (for viruses) and Part 3A. The guidance given in this Annex should not preclude assignment of a particular experiment to a higher standard of containment where this is appropriate.

Naked DNA: possible hazards and routes of transmission

3. Handling naked oncogenic DNA may involve a potential risk to the laboratory worker. Although there is no direct evidence as yet that contact with such DNAs can lead to tumours in humans, this possibility cannot be discounted as evidence does exist for animals.

4. Possible routes of transmission of naked DNA sequences to laboratory workers will primarily be inoculation or entry through broken skin. Other possible routes of transmission such as inhalation, ingestion and eye splashes may be less likely to lead to tumourigenesis.

5. GMSCs should consider any risk to workers in handling oncogenes and related DNA sequences as naked DNA, particularly if linked in a recombinant to strong promoters or enhancer sequences that function in mammalian cells. The GMSC should be satisfied that the laboratory local rules give effective guidance on the maintenance of laboratory discipline and on avoiding accidental inoculation of workers.

6. Work with oncogenes and related sequences is contra-indicated in workers with unprotected skin lesions on the hands or forearms. Where a worker has active eczema, chapping or sepsis, they should consult the BSO before continuing work. It may be necessary to delay a return to work until healing has occurred. Alternatively, the use of suitable personal protective clothing such as gloves and laboratory clothing may be sufficient to prevent exposure. In such circumstances, medical advice may be necessary.

COSHH containment and control measures

7. COSHH regulation 7 requires that exposure to any substance hazardous to health (including a carcinogen) is prevented (e.g. by substitution), or where this is not reasonably practicable, adequately controlled.

8. In cases where the oncogenic DNA is clearly a COSHH carcinogen and it is not reasonably practicable to prevent exposure, Regulation 7(3) of COSHH sets out a series of control measures which must be applied:

- the total enclosure of the process and handling systems, unless this is not reasonably practicable;
- the use of plant, processes and systems of work which minimise the generation, or suppress and contain, spills, leaks, dusts, fumes and vapours of carcinogens;

- the limitation of the quantities of carcinogen in the workplace;
- the keeping of the numbers of persons exposed to a minimum;
- the prohibition of eating, drinking and smoking etc.;
- the provision of hygiene measures etc.;
- the designation of areas which may be contaminated and the use of warning signs;
- the safe storage, handling and disposal etc. of carcinogens ;

9. Full details of the requirements for oncogenes can be found in the COSHH General ACoP, the COSHH Carcinogens ACoP and in HSE Specialist Report No. 41 "Laboratory work with chemical carcinogens and oncogenes"[1]

10. Many of the above measures form a part of normal laboratory practice and are requirements for work with GMOs or biological agents. Given the uncertainty about the hazards of most potentially oncogenic sequences and the small quantities used, prevention of exposure or total enclosure will rarely be "reasonably practicable" (because the costs will outweigh any benefits such as a reduction in exposure). As well as the measures in Part 3A, the measures below, applied in the light of a COSHH assessment, should be used:

a) The importance of good laboratory technique is strongly emphasised. All designated workers should be trained in good laboratory techniques before commencing work with oncogenic DNA sequences. They should be fully aware of the potential hazards of such work.

b) Access to the laboratory where naked oncogenic DNA is handled should be limited to authorised personnel and designated workers.

c) Laboratory benchspace should be designated for work with oncogenic DNA sequences. All designated workers using this space and those likely to be exposed should follow all of local rules for work involving oncogenic DNA.

d) Gloves should be worn for all work with naked oncogenic DNA sequences. Gloves should be chosen taking into account their resistance to any chemicals in use. They should be changed regularly and special attention paid to the danger of glove puncture. Gloves worn for this work should not be worn elsewhere. The use of gloves should not preclude the covering of cuts by suitable dressings.

e) Sharps should be avoided for work with naked oncogenic DNA, except where essential, such as for animal inoculation. Glassware should not be used where plastic alternatives exist.

f) All experimental procedures involving naked oncogenic DNA should be performed so as to minimise aerosol production. Procedures which are likely to generate aerosols such as the use of blenders, sonicators, vigorous shaking and mixing etc. must be conducted under effective engineering controls including suitable local exhaust ventilation systems if appropriate, or in equipment which is designed to contain the aerosol. The suitability of such systems should be decided after a risk assessment as required under the COSHH Regulations. However the control measures utilised for such work must not accentuate the risk in other workplaces or in the outside environment.

g) Where there may be an additional micro-biological hazard, a microbiological safety cabinet (BS 5726: 1992) should be used. Attention is drawn to the guidance in the relevant Annex to Part 3A and the following HSE publications; "An introduction to local exhaust ventilation and guidance" ref. HS(G)37 and "The maintenance, examination and testing of local exhaust ventilation" ref. HS(G)54.

[1] Available from the HSE Public Enquiry Point, Sheffield (tel 0171 892345).

Annex II

MICROBIOLOGICAL SAFETY CABINETS

1. A microbiological safety cabinet (MSC) is a device intended to offer protection to the user and the environment from airborne droplets or particles generated in handling infected and other hazardous biological material. Air discharged from an MSC to the atmosphere is always to be filtered. Two of the three types of cabinet specified in BS:5726: 1992 (see paragraphs 2, 3 and 4) also provide protection against contamination of the product manipulated in them. An MSC is not designed to contain radioactive, toxic or corrosive substances. (See also below under Laminar flow cabinets.) .

2. This Annex merely provides a brief summary of the essentials of the design, function and operation of an MSC. Reference should be made to British Standard 5726:1992 *Microbiological Safety Cabinets,* for a full description of the three types of safety cabinet, Class I, Class II and Class III. The standard also describes the methods for testing air velocity, filtration efficiency and for determining the level of protection provided by them.

3. BS 5726: 1992 is divided into four parts:

Part 1: specification for design, construction and performance prior to installation:

Part 2: recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation:

Part 3: specification for performance after installation.'

Part 4: recommendations for selection, use and maintenance.

4. Parts 1 and the 3 are mandatory if an MSC and its installation are to meet the detailed requirements of the Standard, while Parts 2 and 4 offer useful practical recommendations for safe use. Regulatory authorities may make use of all four parts of the Standard in defining safe working practices, whilst recognising that alternative equipment and procedures may be acceptable, if they provide an equivalent degree of protection.

5. A Class I MSC is designed to provide operator protection by maintaining an inward flow of air past the operator and over the work surface inside the cabinet. As the incoming air is unfiltered, this type of cabinet does not provide product protection. There is a risk that cell cultures, for example, will become contaminated by airborne organisms in the working environment. Class II cabinets, on the other hand, offer protection to both the operator and the product. The inflow of air at the front of the cabinet, which is filtered before circulation within it, discourages emission of airborne particles generated by the work, while the downflow of filtered air over the working surface protects the work. In this model, the design also allows for protection against cross contamination within the cabinet.

6.Class III cabinets (often erroneously called "glove boxes")[1] are totally enclosed units and can provide the maximum protection for the operator, the environment and the work. In this model, both incoming and outgoing air is filtered. Access to the interior of a Class III cabinet is gained by use of armlength gloves attached to ports in the front panel of the unit. Use of Class III cabinets is generally confined to work with biological agents or GMMs assigned to Hazard Group 4 but this model may also be appropriate for other work where the equipment or procedures used may present a risk of vigorous aerosol generation. Furthermore, they may be applicable where there is uncertainty as to the

route of transmission of a hazardous virus, for example, following modification of viral tropism.

7.The minimum inward airflow through the front aperture of a Class I or Class II cabinet is defined in BS:5726. This is necessary to provide containment and is related to the 'operator protection factor' (OPF) for which the minimum is 1.0×10^5. That figure expresses the ratio of the number of airborne particles that would be generated in a procedure conducted on the open bench and the number liberated from the working aperture of a cabinet in which there is the same level dispersal. This means that for every 100,000 particles used in a test as a challenge to the inward flow of air at the working aperture, not more than one should escape. The conditions for conducting the test of OPF are defined precisely in the Standard.

8. Air discharged from all three types of cabinet is filtered before being discharged to atmosphere. MSCs constructed in accordance with BS: 5726 are required to have high levels of filtration efficiency and this is achieved through use of 'HEPA' filters (High Efficiency Particulate Absorption). These are usually made of fan-folded glass-fibre paper with a filtration efficiency of 99.997% when tested by the manufacturer in accordance with BS:3928. In effect, this means that for every 100 000 challenge particles generated in a test of a filter and its seal, no more than three should penetrate.

9.Cabinets should be purchased from a manufacturer or supplier who can show 'type test' certification as required in Part I of BS: 5726 and buyers should be certain to choose a cabinet appropriate for the work. Class I and II cabinets must not be used at Containment Level 4.

10.In general, Class II cabinets should not be used for work with biological agents in Hazard Group 3, particularly where the mode of transmission is through the airborne route. Before a Class II cabinet is selected, the user should consider the agent to be handled, assess the need for protection of the work and relate this to the OPF that can be achieved in the intended conditions of use. Modern Class II cabinets made to the British Standard will provide a protection factor of the same order as Class I cabinets (i.e. a factor of 1×10^5 or better) under the test conditions prescribed in the Standard. However, in day-to-day working condition a Class II cabinet is potentially more susceptible to disruption of it's airflow pattern than is a Class I cabinet. If it can be shown that the required level of operator protection is achieved consistently (as demonstrated by in-use tests - see endnote 2) and provided that the local safety management will allow, a Class II cabinet may be used for some work with certain GMMs (or biological agents) where protection of the work is essential. The cabinet installer should discuss siting with the customer to ensure that the optimum position is chosen consistent with maintaining the required level of safe performance. Factors to be considered are the proximity of the cabinet to doors, windows, ventilation ducts and to movement routes, Once installed, commissioning tests should be conducted to ensure that the safety performance matches that prescribed in BS:5726 and, where appropriate, the standards for product and cross contamination protection.

11.The importance of these commissioning tests cannot be overemphasised. They combine examination of the cabinet's performance and the effects of environmental conditions to demonstrate the level of protection likely to be achieved in practice. When equipment or procedures are being used which might affect the degree of operator protection, appropriate 'in-use'[2] tests should be devised. Safety cabinet containment is sometimes adversely affected by, for example air pressure changes in exhaust ducts and by electrical and/or mechanical problems with control systems where the cabinet exhaust and laboratory ventilation are interlinked. Such difficulties may be overcome by recycling cabinet exhaust air to the laboratory. Because the position of a safety cabinet in a laboratory can be most important in maintaining safe

performance, cabinets that recirculate air should not be regarded as portable. For this reason, fitting wheels or castors to such cabinets should be strongly discouraged. BS 5726 requires that safety cabinets be constructed so as to:

a) exhaust the discharged air to the outside by a dedicated extract system: or

b) recycle discharged air back into the laboratory through two exhaust filters (in such cases the two filters and their seals must be tested independently).

12. Option (a) above is the preferred method. With recirculation (option (b)) there can be problems in, for example, fumigating the cabinet and clearing the fumigant before changing filters etc. Work at containment level 2 does not necessarily demand double HEPA filtration but while filtration efficiency is certainly raised by the use of double filters, the requirement is not so much for this, but rather to provide a 'fail-safe' system in which one filter would compensate for a fault in the other or the seal around it.

13.The choice between total exhaust or recirculation for a particular installation will depend on assessment of local conditions. Recirculation would be inappropriate if a gas or vapour phase of contamination was released in the work process unless, for example, some form of monitored charcoal absorption system was used on the exhaust line.

14.For cabinets that recycle filtered air to the laboratory, it is important to consider in advance safe methods for conducting away fumigant when the cabinet is to be decontaminated. Suitable methods include the use of temporary ducting connected to the air outlet and leading to a fume cupboard.

15.The inward airflow to an MSC, which is drawn through the working aperture of open-front cabinets (Class I and Class II), can be disturbed by, for example, sudden movement of the arms of the operator and turbulence in and around the equipment placed inside the cabinet. A centrifuge, for example, should never be placed inside a MSC unless it is a totally enclosed Class III cabinet. People moving in the vicinity of the cabinet, air movements in the room or changes in air pressure (for example when a door is opened) can also influence it's performance. Disturbances of this kind may significantly affect the level of protection for the operator, particularly when a Class II cabinet is used, because this type generally has a lower inward air velocity through the upper part of the working aperture.

16.Users of safety cabinets must be made fully aware of these limitations and of the way in which safety cabinets operate. More detailed advice on these factors is given in Part 4 of the British Standard.

17.Regulation 9 of the COSHH Regulations in referring to 'local exhaust ventilation', requires a thorough examination and testing of safety cabinet installations to be carried out at intervals not exceeding 14 months, To achieve best practice it is recommended that the procedures in Part 4 of BS: 5726 1992 are followed.

18. In some cases, depending on the frequency of use, regulatory authorities may require a cabinet to be tested at more frequent intervals, for example, six monthly in the case of some containment level 3 and containment level 4 laboratories in order to verify the quality of containment systems in use for a particularly potent biological agent.

19.Fumigation and decontamination of cabinet installations is required before maintenance engineers are allowed to work on the equipment, if they have be used to handle biological agents. Where a cabinet has been used purely to protect the work, for example with much routine tissue culture, the servicing requirements will be purely to maintain a sterile work environment. Similarly, where, for example, plant pathogens are used which are not harmful to humans, there may not be a requirement to service prior to maintenance, however, the environmental risk should be considered. Part 4 of BS: 5726 1992 gives

guidance on suitable decontamination procedures.

20.Although planned maintenance and other checks are a requirement, it will be necessary also to carry out thorough inspection and testing when changes have been made that may affect containment performance. If, for example, a cabinet is moved to a new position in the laboratory, full commissioning tests will be needed. Other changes such as placing equipment around or near the cabinet may require less stringent checks.

21.So-called 'laminar flow' cabinets are NOT microbiological safety cabinets and should never be used when handling infectious or potentially infectious materials. Laminar flow cabinets are designed to deliver a stream of HEPA filtered air across ('horizontal laminar flow') or down onto ('vertical laminar flow') a working surface so as to provide an environment in which sterile materials such as culture media, drug preparations do not become contaminated. Their mode of operation is such that any airborne droplets generated in, for example, pipetting and similar manipulations are actively directed at the operator. The use of laminar flow cabinets with any material infectious or potentially infectious for humans is therefore positively hazardous.

[1] A 'glove box' is simply a box (usually made of transparent plastic or with a plastic or glass window) with gloves attached but not necessarily with any throughput of air filtered or unfiltered. Glove boxes are a primitive form of containment and are not generally suitable for handling infectious materials

[2] The "in-use test" referred to is the operator protection factor measured according to the procedure in BS:5726, but with the artificial arm removed. In it's place, an operator works with hands and arms within the cabinet throughout the test, and performs, for example, typical repetitive pipetting procedures. Other in-use tests may also be necessary, based on the actual conditions and work practices of individual laboratories.

Guidance on technical issues and the use of safety cabinets is available from the Health and Safety Executive, Dangerous Pathogen Unit, Magdalen House, Stanley Precinct, Bootle, Merseyside, L20 3QZ

Annex III

CHEMICAL INACTIVATION OF GMMs

1.The Contained Use Regulations require that, for certain operations, waste is inactivated prior to discharge. For large scale operations this will often involve heat inactivation of cultures, however, one of the main approaches taken at laboratory scale is the use of disinfection. The regulations require that cultures are inactivated by a validated means. Where chemical disinfection is used, this means that the method used should be validated (or verified) under working conditions. This is important, because the presence of, for example, organic matter, can seriously affect the performance of certain disinfectants. Similarly, where cell cultures are used under buffered conditions, in the presence of proteins, the disinfection regime may be compromised.

Definitions

Disinfection:Disinfection generally refers to the use of chemical agents to destroy the potential infectivity of a material, but does not imply the elimination of all viable micro-organisms. Effective disinfection is dependent upon:

Activity: the effectiveness of a particular disinfectant varies with the target micro-organism.

Concentration: the 'use-dilution' is the correct concentration for effective disinfection in particular circumstances, eg spillages, discard jars. The effective conentration may be dependent upon the age of the solution, as once diluted some disinfectants lose effectiveness with time.

Contact: intimate contact for a sufficient period of time must be maintained between the disinfectants and the contaminated article, eg air bubbles should be removed from submerged articles.

2.The disinfectants in most common use are hypochlorites, chlorine releasing agents, phenolics, alcohols, aldehydes and surface-active agents, quaternary ammonium compounds, peroxygen compounds:

Hypochlorites eg, Chloros, Domestos:

3.Hypochlorites have a wide spectrum of antimicrobial activity and are rapid in action but they are corrosive, inactivated by organic matter and decompose once diluted.

Chlorine releasing agents (e.g. sodium dichloroisocyanurate - NaDCC)

4.These are broad spectrum and rapid acting biocides, but they are corrosive and easily inactivated by organic matter. Tablets or granules of NaDCC are stable when stored dry and dissolve in water to give available chlorine. Chlorine releasing agents are widely recommended in conditions where corrosion or bleaching are not a problem. The rate of chlorine generation is accelerated in an acidic environment.

Phenolics eg Hycolin, Stericol, Clearsol

5.Phenolics are non-corrosive and have a wide range of activity but may be ineffective against non-lipid viruses. They are not readily affected by organic matter, but their antimicrobial activity may also be reduced by hard water. Phenolics should not be stored diluted.

Alcohols eg 70% ethanol, 60% isopropanol

6.Alcohols give a very rapid kill of bacteria and some viruses, but because they are relatively volatile do not provide a sustained antimicrobial action. Alcohols are flammable and require appropriate precautions in storage and use. They should not be used in microbiological safety cabinets or on large areas.

Aldehydes

7.Formaldehyde as the vapour or the aqueous solution (formalin) is toxic and is not suitable for general purposes. However, it is used for fumigating microbiological safety cabinets and certain rooms (eg high containment laboratories). During fumigation, containers of other disinfectants should be sealed if the disinfectant is incompatible with the fumigant. Glutaraldehyde eg Cidex, Tegodor, is also toxic but has a relatively low vapour pressure and is usually used as a solution. It has a wide range of activity, including against bacterial spores. It is non-corrosive, but does not readily penetrate organic matter and is not particularly stable once activated.

Surface-Active Agents (Quaternary ammonium compounds, QACs)

8.QACs are fungicidal and bactericidal (less so against gram-negative bacteria, but not sporicidal or tuberculocidal, and they show variable activity against viruses. They are inactivated by soaps, anionic detergents and organic matter. They are often sold in combination with other disinfectant groups.

9.Only the cationic and amphoteric detergents have any antimicrobial activity, and these are regarded as being more bacteriostatic than bactericidal. They are relatively non-toxic and non-irritant. QACs form the basis of the majority of cationic detergents, eg Cetrimide, Roccal. Only a limited range of amphoteric detergents have been produced as antimicrobial agents, eg Tego.

Iodophors

10.Iodophors are combinations of iodine and a solubilising agent or carrier providing a sustained-release reservoir of iodine. They are bactericidal. fungicidal and virrucidal but show limited activity against spores, are inactivated by organic matter (dependent upon preparation and concentration) and may corrode metals.

Peroxygen compounds

11.Peroxygen compounds (e.g. Virkon) have a wide range of microbial activity although this is reduced by organic matter. Uses include routine laboratory disinfection, but before using on equipment approval should be obtained from the manufacturer of the equipment since corrosion of some metals may be a problem.

Sodium hydroxide solutions

12.Sodium hydroxide solutions (1%) may be used under certain circumstances as a laboratory disinfectant. Care must be taken with their use, due to the caustic nature of the chemical. It is often used to inactivate prions, as it is less corrosive than concentrated hypochlorite solutions. This is particularly true when steel (or other metal) surfaces require disinfection.

Selection and Use

13.When selecting a disinfectant its toxicity to man and the appropriate health and safety precautions should be considered. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed.

14.Arrangements should be made for appropriate procedures and training to ensure that suitable disinfectants, at the correct dilution are available at the point of use. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor not only the performance of a particular disinfectant but also the way in which it is used, for example to detect dilutions wrongly made up

or not made up freshly, the use of dirty containers and in compatible reagents for example, certain types of detergent.

Annex IV

FUMIGATION

1. `On occasions it will be necessary to decontaminate laboratories, animal containment facilities and safety cabinets by fumigation when, for example, there has been a spillage of infectious material or when servicing or maintenance work is to be carried out. Fumigation should always be a planned exercise with appropriate controls in place and with information and warnings provided for those who need to know. Fumigation operations should only be carried out by named, trained personnel working to an agreed plan and using a method that is known to be effective in the circumstances of use.

2. Formaldehyde vapour has been known for many years as a highly effective biocidal agent, is the fumigant most commonly used in laboratories. There is more than one way of generating formaldehyde but the usual source is formalin which is readily available as a 40% solution of formaldehyde vapour in water. When heat is applied, large quantities of the vapour is generated. (See endnotes 1 and 2.)

3. For formaldehyde to act to maximum effect, it must be able to penetrate (hence pre-cleaning is helpful if it can be done without jeopardising safety) and, it must be able to dissolve at adequate concentrations in the film of moisture in the immediate vicinity of the organisms to be inactivated. Water vapour generated in the process of dispersing formaldehyde (see paragraph 4) provides the essential optimum level of relative humidity (ie greater than 35% but less than 80%). Too much formaldehyde results in the deposition of sticky deposits of paraformaldehyde.

4. There are a number of methods of generating formaldehyde vapour: heating a mixture of formalin and water in a thermostatically controlled heating unit (such as an electric frying pan or electric kettle); mixing formalin and water with potassium permanganate crystals*; using commercially available formaldehyde generating kits; and, heating formalin in a purpose-made vaporising unit (safety cabinets).

> * WARNING: the correct relative concenration of these two components is essential to avoid a violent reaction.

5. Microbiological safety cabinets should always be fumigated if a large spillage of infectious material occurs within them, before filters are changed or any maintenance work is carried out which involves gaining access to the interior of the cabinet (for example air ducts). Fumigant should be generated with the night door securely sealed and the non-return valve left closed. Passive migration of the fumigant through the filter can occur but an alternative is to leave the valve open and the fan running for 10 to 15 seconds to ensure penetration of the filter medium. The valve should then be closed and the fan switched off while the remainder of the fumigant is left to disperse within the cabinet. After at least six hours, or preferably overnight, the fumigant should be exhausted to atmosphere by switching on the fan and allowing air from the room to enter the cabinet (for example through a large bung-hole in the night door). Before venting the formaldehyde in this way, it is essential to ensure that no-one is in the vicinity of the exhaust outlet and that the exhaust air does not enter nearby windows or ventilation air intakes.

6. If filters are to be changed after fumigation, the discarded filter unit should be bagged and autoclaved before disposal. There are special difficulties if the cabinet is used with the agents causing transmissible spongiform encephalopathies as they are resistant to inactivation by formalin. More detailed advice on the fumigation of safety cabinets is given in Part 4 of British Standard 5726:1992

Fumigation of rooms

7. Where a room in a laboratory or animal containment unit is to be fumigated the area should be checked to ensure that it is securely sealed so as not to allow the escape of fumigant to other parts of the building. Suspended ceilings can present a special difficulty as there may be a void above connecting with other rooms nearby.

8. It should be noted that any hydrochloric acid and chlorinated disinfectants should, if possible, be removed from the room before fumigating with formaldehyde. This is to prevent the possibility of forming bis (chlormethyl) ether which may be carcinogenic. In high containment facilities, care must be taken where double ended dunk tanks are present.

9. A test of the effectiveness of the fumigation may be carried out by placing spore strips/discs carrying *Bacillus subtilis* var. *globigii* (filter paper inoculated with a suspension of the organism) at various points in the room to test penetration of the fumigant. Similarly, a standardised spore suspension may be painted onto small marked areas on surfaces which are later swabbed to recover any surviving organisms.

10.Exposure to the residual effect of the fumigant after generation should be for at least six hours or preferably overnight. Fumigant may be extracted from the area by the air handling system but only when that is a total loss system with no possibility of formaldehyde vapour being conducted to other areas. More commonly, use is made of a microbiological safety cabinet or a fume cupboard as a means of extraction if one is situated within the area under treatment and if it, exhausts to atmosphere. In all cases the plant or equipment extracting the air should be operated by an external switch so as to avoid entering the room.

11.After the fumigant has been evacuated in this way, there should be a thorough check of the level of residual vapour before anyone enters. This may be done most conveniently by, for example, sampling the air through a small port fitted in the door for this purpose. Meters and other assay devices are available to indicate the concentration of formaldehyde vapour remaining in the air. (See endnote I.)

12.A number of factors affect the efficiency of fumigation. The ratio of formalin to water used and thereby the relative humidity created, the volume of the space to be fumigated, the surface area exposed in that space and the presence of absorbent materials such as cardboard boxes. At temperatures below 18°C formaldehyde fumigation is less effective. Below 9°C, formaldehyde sublimes and is less easy to vaporise.

13.Personnel should not enter an area when a major spillage of micro-organisms has taken place as there may be a great risk of exposure to infection by organisms that may remain suspended in the air for some time. Moreover, personnel should not enter an area when the fumigant has been generated except in a dire emergency when full breathing apparatus which provides air from an independent source must be worn. Only those trained in the use of breathing apparatus should use it. Respirators are not appropriate for use in the concentrations of formaldehyde vapour achieved when carrying out these procedures.

Notes

1 Formaldehyde is a Schedule 1 chemical under the COSHH Regulations and has a *Maximum Exposure Limit (MEL) of* 2 ppm or 2.5 mg m^{-3}

2 Formaldehyde vapour is explosive at 7.75% in dry air. It's ignition point is 430°C

In case of difficulty, HSE's Biological Agents Unit (Magdalen House, Stanley Precinct, Bootle, Merseyside L20 3QZ) is able to provide advice on fumigation.

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